INVESTIGATION OF SEROLOGICAL EVIDENCE

A Manual for Field Investigators

SUSAN M. HUNT, B.S.E., M.A.J.

Forensic Chemist

Written by a forensic chemist for uniformed officers and field investigators, this text presents the latest methodology for the field investigation, analysis, and individualization of serological evidence. The sequential organization of chapters allows the reader to progress from simple to complex concepts — from the officer's initial contact with serological evidence to the phases of analysis. In the first section of the book, the author focuses on field investigation, and she covers recovery techniques, identification at the crime scene, interpretation of bloodstains, and collecting and packaging serological evidence. The second section focuses on laboratory analysis and describes techniques of identification, origin determination, and individualization of serological evidence. An appendix details roll call training techniques for reviewing and reinforcing the material presented.

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By

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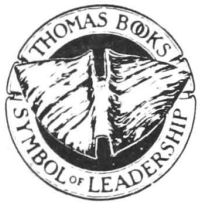

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To my mother, Martha Hunt, for her constant support
and encouragement throughout my career.

PREFACE

FOR the past several years while working as a forensic chemist with a metropolitan criminalistics laboratory, the author has seen many significant criminal cases brought to a successful conclusion through the careful efforts of uniformed officers, whose early arrival at the crime scene and professional evaluation, collection, and preservation of serological evidence made the difference between a good, prosecutable case and no case at all. Unfortunately, the forensic literature currently available to the uniformed officer or field investigator has fallen far behind many important new advancements in forensic serology.

Concurrently, as a result of court decisions that call for increased utilization of scientific evidence and that place serious restrictions on traditional methods of police interrogation, the criminal justice establishment has placed ever increasing emphasis upon improvements in physical evidence processing and scientific analysis of evidence.

The Investigation of Serological Evidence attempts to bridge this gap by presenting the latest techniques developed for the field investigator, analysis and individualization of serological evidence. Though scientifically and academically sound, this manual is written in easily assimilated language at pedagogically established reading levels appropriate for the uniformed officer and field investigator.

The Investigation of Serological Evidence is divided into three major components: the field investigation of serological evidence, the analysis of serological evidence, and the individualization of serological evidence. It is further divided into chapters that begin with the officer's initial contact with serological evidence at the crime scene and progress through the various phases of analysis pertinent to that evidence. This organization permits the reader to progress from the simple concepts of discovering and identifying serological evidence to the more complex concepts of individualizing that evidence. This organization is consistent with the most fundamental pedagogical principle as it involves prescribing ideas and facts familiar to the reader and building upon them to develop new concepts.

To insure maximum utilization of *The Investigation of Serological Evidence* by

the uniformed officers and field investigators for whom it is primarily intended, the author undertook a study of readability levels of police officers in the United States. The Flesch Reading Ease and Dale-Chale Readability formula were used in conjunction with several studies of police educational achievement levels to establish the readability level of this manual (40 to 50 on the Flesch Readability Ease score). A glossary is included to facilitate the reader's understanding of essential but unusual terminology. Selected roll-call training is included to facilitate review and reinforcement of the material presented as well as to enhance the manual's usefulness in the training environment.

Review and field testing of *The Investigation of Serological Evidence* in small, medium, and large city police departments and at the Consolidated Federal Law Enforcement Training Center has resulted in unanimous statements of praise for the forensic value and practical usefulness of this manual.

The Investigation of Serological Evidence, which was developed initially from a master's practicum prepared under the supervision of the Department of Criminal Justice, Wichita State University, owes much of its value to the following individuals who dedicated their time and energy to evaluating this work: Police Officer Marty C. Ingram, Overland Park (Kansas) Police Department; Lieutenant M. M. Wasson, Los Angeles (California) Police Department; Mr. Morris Grodsky, Forensic Science Specialist, Consolidated Federal Law Enforcement Training Center; Chief Inspector Edward Scheu, U.S. Marshal's Training Academy; and Sergeant Merrill D. Rice, Homicide Unit Supervisor. Special recognition is given to Chief Inspector Reis R. Kash, U.S. Marshal Service, for his constant dedication, support, and guidance in this effort.

INTRODUCTION

HUMAN law is essential to American society, and forms the basis of our democratic system of government. A democratic system of laws is necessary to preserve individual freedom and prevent acts that infringe upon the freedom. To insure obedience to the law there must exist a mechanism of enforcement. For this reason federal, state, county, and city law enforcement agencies have been established and charged with the responsibility of upholding the criminal laws applicable to their jurisdictions.

Without the effective enforcement of these laws the democratic principle of individual freedom would be severely impaired.[1] To carry out this function effectively, the law enforcement officer must fulfill the role of a criminal investigator. The purposes of criminal investigation are as follows:

1. Establish the fact that a violation of the law has been committed.
2. Identify and apprehend the individual or individuals responsible for committing the violation.
3. Assist the prosecutor in presenting the case against the defendant in a court of law.[2]

The responsibilities of a law enforcement officer involve a lawful search for people and physical objects useful in determining that an illegal act has been committed, and in reconstructing the facts of that act. Criminal investigation involves proceeding from the known to the unknown, a process that works backward in time to ascertain the truth concerning a crime in question. The ultimate goal of this process should be the development and proof of all relevant facts concerning the commission of a crime. This should lead to the successful prosecution of the suspect in court or the establishment of the innocence of persons wrongfully accused.

The criminal investigation of a crime by a law enforcement officer can be divided into two major areas:

1. A search for verbal information concerning the facts of the crime from the victim, witnesses, possible suspects, and informants.
2. A search for the physical evidence of a crime in order to support statements of victims, and the development of a theory describing the events as a guide for further investigation.[3]

This manual is concerned with the second area of investigation, physical evidence.

Physical evidence has been present at crime scenes since the beginning of investigative law enforcement. However, it was often ignored due to the lack of recognition of its potential, the inability to properly preserve and collect it, and lack of capability to interpret its significance and value to the investigation.[4]

The introduction of the techniques of science into law enforcement, with the development of the criminalistics laboratory, began shortly after World War I, when American police officials realized how far behind their European counterparts they were in scientific investigation techniques.[5]

The practice of incorporating scientific techniques was promoted by Hans Gross, whose basic thesis later became the keystone of modern criminal investigation; "the scientific method in reconstructing a crime is necessary, possible, and likely to be a decisive factor in solving a case."[6]

Successful criminal investigation requires innovative thinking and diligence on the part of the investigator and a close working relationship with the forensic scientist in the laboratory. The investigator and the forensic scientist should work together as a team in reconstructing the events of a criminal offense and in the identification of the suspect or suspects responsible.

The recognition and collection of physical evidence has not kept up with the recent advances in the scientific analysis of that evidence. What is needed to bridge this gap is the scientific training of law enforcement officers in the recognition, identification, and collection of physical evidence.

One of the important types of physical evidence that can be associated with a serious crime is serological evidence. Serological evidence consists of blood and body fluids found at the scene of the crime. Benjamin Grunbaum gives the following analysis of the potential of serological evidence to the investigator:

> Blood shed by the victim can yield valuable information to the crime investigator. The discovery of unexplained blood or bloodstains may be the first indication that a crime has been committed. Some of the victim's blood may be carried away on the person or clothes of a criminal, or bloodstained weapons may come to be associated with a suspect. The pattern of blood stains and, if there is more than one victim, the identification of each bloodstain, may assist in reconstruction of the crime. Bloodstain evidence may be used to corroborate or refute statements of persons involved in a case, or it may exculpate a suspect.[7]

The great amount of research that has been done with respect to serological evidence has resulted in an increased number of techniques available to analyse blood and body fluid stains associated with a serious crime.[8] In the 1970s the usual determinations made by a forensic serologist included determinations of species origins, ABO blood group, and the identification of blood and semen stains.[9] With the increase in knowledge and the development of new techniques, a long list of genetic markers found in blood and body fluids can now be identified. These genetic markers allow for a greater probability for associa-

tion of specific persons with events and for excluding individuals from suspicion. However, none of these techniques can be applied to physical evidence associated with a crime unless the investigator has an understanding of the capability of the criminalistics laboratory and a clear knowledge of how to collect and preserve the evidence. Paul Kirk, noted criminalist, has stated:

> It can be stated categorically that more laboratory failures are due to inadequate collection of the existing evidence than are caused by the failure of the laboratory to examine it properly.[10]

This situation is further summed up by Benjamin Grunbaum:

> The ultimate value of blood evidence to the criminal justice system rests with the law enforcement personnel and attorneys, who must understand both the possibilities and limitations of bloodstain analysis. They must know of the services available to them in the crime laboratory and how to put them to good use in support of investigative effort and in presentations in courts of law. [1]

For these reasons this manual is written specifically for the use of law enforcement officers as an aid to help them properly identify, evaluate, preserve, and collect serological evidence and to gain a general understanding of the theories and techniques employed in the crime laboratory in the analysis of that evidence.

This manual is divided into two parts. Section I deals with the investigation of serological evidence by the officer at the crime scene. Section II discusses the common types of analyses of evidence that the crime laboratory is capable of performing.

CONTENTS

INVESTIGATION OF SEROLOGICAL EVIDENCE

SECTION I

THE FIELD INVESTIGATION OF SEROLOGICAL EVIDENCE

Chapter 1

GENERAL TECHNIQUES USEFUL FOR THE RECOVERY OF PHYSICAL EVIDENCE

THE NATURE AND VALUE OF PHYSICAL EVIDENCE

PHYSICAL evidence includes any and all objects that can establish that a crime has been committed, or provide a link between a crime and its victim, or a crime and the suspect. There are two purposes for studying the physical evidence found in conjunction with a criminal offense:

1. Physical evidence often represents the decisive factor in determining the guilt or innocence of a suspect.
2. Physical evidence can be a material aid in identifying the suspect.[12]

Not only is physical evidence admissible in court, but its use has been encouraged. The United States Supreme Court has ruled several times on the importance of physical evidence in criminal trials.[13] The increased reliance on physical evidence by the courts and the police is due to the fact that physical evidence is not subject to lapse of memory, confusion, or perjury, as are human witnesses.[14]

The physical evidence of a criminal offense can generally be recovered from three sources:

1. The scene of the crime
2. The victim, if any, of the crime
3. The suspect, and his environment[15]

Once physical evidence is recovered by an investigating officer, it should be sent to the crime laboratory for analysis. The type of analysis and the identifications possible by the crime laboratory is determined by the individualizing characteristics of the item of evidence. Physical evidence can be grouped into two general categories based on the individual characteristics it possesses:

1. *Physical Evidence with Class Characteristics Only.* A definite identification of this type of evidence can never be made, due to the possibility of more than one source of origin. Examples of this type of evidence include; single layer paint samples, soil, glass, fibers, hair and blood.

2. *Physical Evidence with Individual Identifying Characteristics.* This type of evidence can be positively identified, with a person or a source, if sufficient accidental or microscopic markings are present on the item. Examples include; fingerprints, handwriting, bullets, and tool marks.[16]

An investigator should never overlook an item of physical evidence even though it possesses only class characteristics. Every item of physical evidence located at a crime scene should be recorded and recovered by the investigator. The collection of too much evidence is obviously better than collecting too little. However, the collection of irrelevant items may cloud the investigation. For this reason, training and knowledge are necessary for the investigator properly to recognize and collect evidence at the crime scenes.

This manual will concern itself with only one form of physical evidence: serological evidence. This type of evidence can be found in conjunction with almost any crime but is most often found at the scene of violent crimes against persons. Serological evidence recovered in association with a criminal offense can potentially—

1. link the scene or the victim with the suspect;
2. establish an element of the crime;
3. corroborate or disprove an alibi;
4. induce an admission or confession;
5. exonerate the innocent.[17]

GENERAL TECHNIQUES OF CRIME SCENE SEARCHING

"The first step in utilization of bloodstain evidence is the systematic processing of the scene of the crime by trained technicians."[18] The crime scene represents the physical location where the suspect either committed the crime or left physical evidence of the crime.[19] The purpose of searching this area is to recover any physical evidence that may link the suspect with the scene, or victim, and to reconstruct the events of the crime.[20] In conducting a crime scene search, the goal of the investigator should be to accomplish a comprehensive and nondestructive accumulation, within a reasonable period of time, of all available physical evidence.[21]

In conducting this search the investigator should always be aware of the following legal requirements of physical evidence:

1. The investigator must be able to identify, in court, each piece of evidence collected.
2. The investigator must be able to describe, in court, the exact location of each item recovered.
3. The investigator must be able to prove, in court, that a proper chain of custody has been maintained on each item submitted into evidence.
4. The investigator must be able to describe and account for any change

that may have occurred in the evidence between its collection and its introduction in court.[22]

Upon arriving on the scene of a crime, the investigator should immediately secure and protect the area. This step is essential to preserve the integrity of the evidence present and to insure that nothing will be tampered with before it can be properly recorded and recovered.

After the scene is secured, a preliminary examination of the area should be conducted. During this examination the investigator should look for items, conditions, and locations that potentially contain the most information. The actions of the investigator at this stage should consist only of observation and recording of the evidence present. Photographs of all items, as well as photographs depicting the relationship between the items should be taken. During this preliminary examination the Golden Rule of Hans Gross should be the guiding principle of the investigator; "never alter the position, or pick up, or even touch any object before it has been minutely described in an official note and a photograph taken."[23] Further discussion on the sketching and photographing of serological evidence will be presented in Chapter 4.

After this preliminary examination is finished, and the scene has been thoroughly photographed, physical evidence can then be recovered. Each crime scene presents a unique challenge to the investigator. However, there are some areas common to all scenes that should be recognized and searched:

1. The method of transportation used by the suspect.
2. Place of entry. In a forced entry the suspect may have been injured and left traces of blood.
3. Suspect's path through the scene. The investigator must use logical thinking and note any disarray of furniture in determining this pathway. The purpose of making this determination is to limit, to some extent, the area to be concentrated on for intensive searching. This will concentrate efforts in those areas that present the greatest probability for finding physical evidence.
4. Contact of the suspect with the scene or the victim. This area could possibly contain transfer evidence from the suspect to the scene or victim. This transfer may include blood, semen, and saliva. The greatest possibility for the transfer of serological evidence occurs in violent crimes against persons. This possibility should not be overlooked in property crimes where the suspect was injured.
5. Place of exit. All doors and windows should be thoroughly examined for traces of blood.[24]

Chapter 2

THE IDENTIFICATION OF SEROLOGICAL EVIDENCE AT THE CRIME SCENE

INTRODUCTION

BLOOD is one of the most important and most frequently encountered types of evidence in criminal investigation."[25] Blood can be found at the crime scene or on the victim, the weapon, and possibly the suspect. Other types of serological evidence that can be recovered from the scene include semen and saliva. These types of evidence are most often found in association with violent crimes against persons, but their presence should not be overlooked at other types of crimes.

This chapter will present general characteristics of the three most common types of serological evidence encountered by the investigator—blood, semen, and saliva—and some general techniques useful in recognizing and identifying this evidence at crime scenes.

GENERAL CHARACTERISTICS OF BLOOD

Blood is a fluid (normally red in color) that circulates throughout the body, transporting oxygen, nutrients, and waste materials. The volume of blood of an average human being is six to eight quarts and accounts for 6 percent to 8 percent of the total body weight.[26] If a tube of whole blood is allowed to stand, with the addition of a chemical to prevent clotting, it will separate into two distinct parts. The upper yellow liquid portion is called the plasma, and the bottom solid red portion contains the blood cells.

The plasma, or liquid portion of the blood, makes up 55 percent of the total blood volume.[27] Plasma is composed of approximately 90 percent water, the source of which is food and the water produced by metabolism.[28] This supply of water is adjusted by elimination through breathing and through the excretory system. By these processes the total water content of the body, and the volume of the blood, is kept under control.[29] The materials carried by the plasma

include glucose (blood sugar), hormones, vitamins, proteins, minerals, the chemicals necessary for blood clotting, and the blood cells. Some of the proteins contained in the plasma are called antibodies. These antibodies are useful in forensic identifications and will be discussed further in Section II of this manual. Serum is a term used to describe blood plasma that has had all of the chemicals used in blood clotting removed.

There are three types of cells contained in blood: the red blood cells, or erythrocytes, the white blood cells, and the platelets. Red blood cells are produced in the bone marrow and are disc shaped with indentations on each side. A cubic millimeter of blood contains approximately 5 million red blood cells.[30] The primary function of the red blood cells is to transport oxygen from the lungs to the body tissues and then transport carbon dioxide produced as a waste product back to the lungs, where it is exhaled. This function is accomplished by a chemical, called hemoglobin, which is contained in the red blood cell. This chemical is also responsible for the red color of blood.[31] Hemoglobin is composed of two parts, a pigment containing iron, heme, and a protein component, globin.

The major function of white blood cells is to protect the body against infection.[32] Leukocytes, one type of white blood cell, are capable of moving through the walls of the blood vessels and traveling to the site of an infection. Once they have reached the site of an infection, they will engulf and destroy any bacteria present. The accumulation of white blood cells at the site of an infection is called pus.[33]

Platelets are cell fragments formed in the bone marrow.[34] These platelets will rupture on the edges of a torn blood vessel and initiate the blood clotting mechanism. Upon rupture, the platelets release a chemical, which in combination with other chemicals found in the blood, will form a blood clot. The actual blood clot is formed from a protein in the plasma called fibrin. Fibrin forms a tangled network of long strands, which traps blood cells. This fibrin network covers the wound opening and prevents excessive bleeding. A pure blood clot is yellowish-white in color, although the fibrin network traps red blood cells giving the clot a red color.[35]

Figure 2.1 represents a microscopic view of the components of human blood.

LOCATING BLOODSTAINS AT THE CRIME SCENE

In searching for bloodstain evidence at the crime scene, the investigator should rely on the circumstances and any information available concerning the crime. As a general rule, any blood that has been shed will be visible to the investigator.[36] This may be in the form of large pools of liquid blood or smaller dried stains. Whenever large liquid pools of blood are observed, they should be protected until they can be properly photographed and collected.[37] This can be

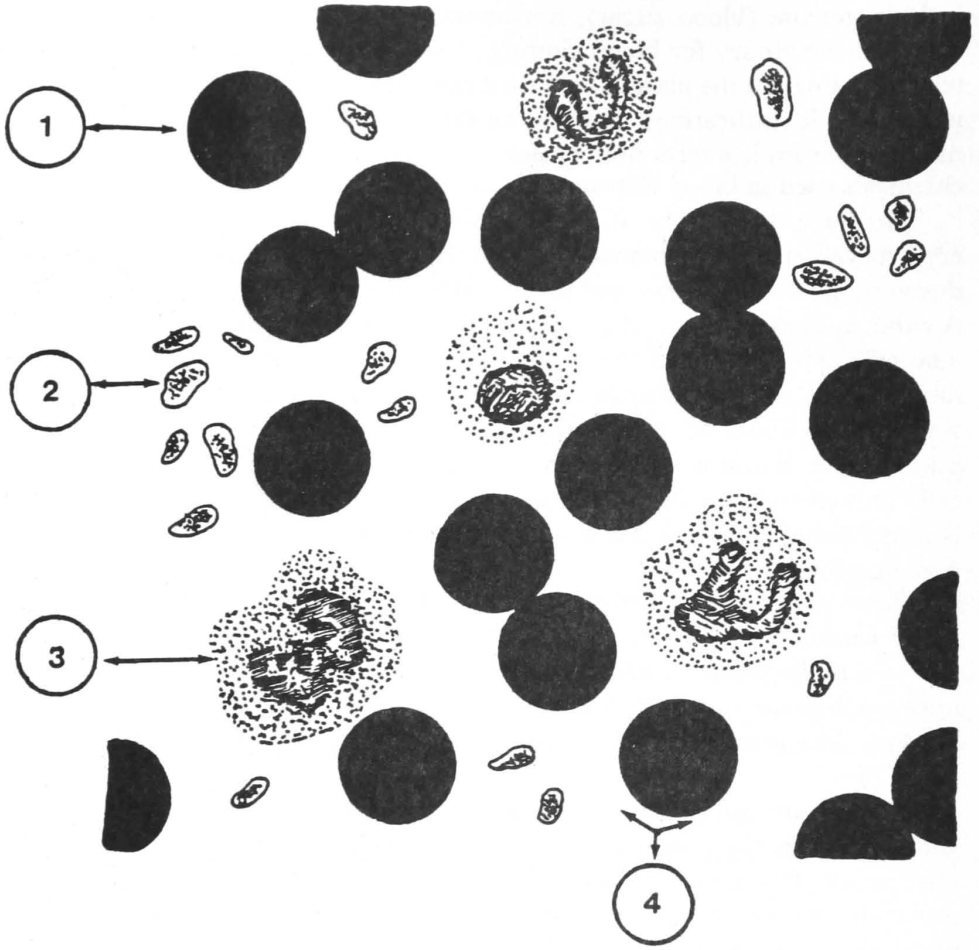


Figure 2.1. A microscopic view of the components of human blood. Key: 1—Red blood cell. 2—Platelets. 3—White blood cell. 4—Plasma.

accomplished by covering them with a glass, pan, or some other suitable object. This protects against an officer inadvertently stepping in the blood and tracking it to other areas of the scene. Large liquid pools will usually be present only in situations in which large amounts of bleeding occurred and the investigator has arrived shortly after the incident occurred. Most often blood will be in the form of dried stains located on the victim, on the suspect, if present, and possibly on any object located at the scene. Special attention should be given to deposits that are separated from the general deposits, as they may be from a different source, such as the suspect.

Relatively fresh, dried bloodstains will have a reddish-brown glossy appearance.[38] However, the effects of sunlight, heat, wind, and water may cause them to appear grey in color. The time required for this change in color is dependent on the environmental conditions and the surface the blood is deposited on.

Metal surfaces will cause a quicker color change than fabric.[39] Bloodstains found on wallpaper may exhibit surprising colors due to the absorption of the dyes from the paper. The appearance of blood on fabrics will vary with the type and content of the material. Blood will soak completely through some fabrics, while on others it will remain on the surface. The glossy appearance of blood is also less pronounced on most fabrics. Because of the varied conditions encountered at the crime scene and the variety of objects on which blood may be deposited, other colors from red to black, and green, blue, or greyish-white, may be observed.[40]

The determination of the age of a bloodstain is often very difficult, if not impossible.[41] This determination can only be made in the crime laboratory, though useful information concerning the age of bloodstains can sometimes be obtained by studying the degree of dryness of a stain.[42] If the stain is very fluid, it is probably less than one hour old, though this is dependent on the size of the pool. Blood that has the consistency of gelatin indicates that fibrin has begun to form. A simple test for this is to draw a pencil line through the stain. If fibrin is formed, the line will remain visible after the pencil is removed. On further drying, the stains will contract, and the edges will begin to crack and pucker.[43] The time necessary for any of these changes depends on the conditions, such as the temperature and humidity of the surroundings. Some estimate, however, as to the elapsed time that can be made from the appearance of the stain.

A technique that is sometimes helpful in locating less obvious stains is to search with a flashlight. If the light from a flashlight is allowed to fall obliquely (at a slanted angle) against the surface being examined, bloodstains will sometimes show up better.[44] Another technique involves the use of colored lights. Illuminating the area with red light and then green light can sometimes be helpful in locating bloodstains.[45]

Luminol is a chemical reagent that is very useful in locating small traces of blood at a crime scene. The only requirement for the use of luminol is that the scene be completely dark. Luminol is a chemical that when sprayed on a surface will react with any blood present, causing a luminescence in a dark room. Any luminescence observed is only an indication that blood may be present, not a positive identification, as other materials may give the same luminescence.[46] Luminol is prepared by mixing sodium perborate, 3-aminophthalhydrazide, and sodium carbonate in a ratio of 0.14: 0.02: 1.[47] These dry chemicals are then mixed with water (at the crime scene), the room is darkened, and luminol is sprayed on the surface to be examined. A strong luminescence is indicative of the presence of bloodstains. Luminol should never be mixed or sprayed in metal containers. This chemical will sometimes luminesce in the presence of metals.

The area surrounding the victim of a homicide, or the area of violent suspect-victim contact, should be thoroughly searched for bloodstains. The

clothes and the body of the victim should be examined. The investigator should not overlook the fingernails and cuticles of the victim's hands, as these areas may contain traces of the suspect's blood left as a result of a struggle. A common practice in homicide investigations is to place the victim's hands in paper bags so fingernail scrapings can be obtained at the morgue.[48]

Any possible weapons discovered should be examined. It does not, however, follow that blood has to be found on a knife or similar object for it to be the weapon.[49] The edges of the wound could have wiped off the blood, or it may have been cleaned by the suspect.

Once the immediate area of suspect-victim contact has been searched, attention should be focused on other areas of the scene. Bloodstains in areas not easily correlated with the victim may possibly belong to the suspect and should be properly recorded and collected.[50]

Occasionally the assailant will attempt to clean up the scene. Furniture will be straightened, and blood will be washed off in an attempt to conceal the crime, destroy evidence, or to delay the discovery of the scene.[51] The crime scene search in this case should be extended to areas not in direct view. Blood that has been shed on a wooden, tile, or linoleum floor and then washed can usually still be found in the cracks, joints between tiles or wooden planks, or under the corners of the linoleum, as these areas are somewhat resistant to washing attempts.[52] Bloody hands will usually be cleaned at the first opportunity. If a sink is used for this purpose, traces of diluted blood will remain in the trap of the drain for a considerable length of time.[53] If this could possibly have occurred, the drain should be disassembled, and the water from the trap collected and sent to the crime laboratory for analysis. The underneath part of the faucet should be checked for blood traces the suspect may have overlooked in cleaning.[54] In the absence of washing facilities, the suspect will usually wipe his hands on something to remove the blood. The natural inclination is to use something other than one's own clothing.[55] For this reason the bottom of furniture, underside of rugs, curtains, and any towels or rags should be examined for blood. All trash cans, garbage pails, and clothes hampers should be searched for bloody towels or rags.

On rare occasions the suspect is apprehended at the crime scene. This situation affords the investigator the perfect opportunity to examine and collect any clothing containing bloodstains, semen, or other physical evidence.

FIELD TESTS FOR BLOOD IDENTIFICATION

There are chemical tests that the investigator can use for the presumptive identification of blood at the scene. These tests are called presumptive tests because none of them are specific for blood and therefore cannot be used as a positive identification. A positive result indicates that blood may be present,

and the stain should be sent to the crime laboratory for further analysis. A negative result, on the other hand, indicates that blood is not present, and no further testing is necessary.[56]

The benzidine, leuco-malachite green, and luminol can all be employed at the crime scene for the presumptive identification of blood. The luminol test was discussed previously, so this discussion will concern itself with the benzidine and leuco-malachite green tests. These two tests are based on the activity of the heme group.[57] As discussed earlier, heme is a component of hemoglobin, which is a chemical present in human blood cells. This heme group exhibits what is called peroxidaselike activity.[58] Peroxidase is an enzyme that can speed up a chemical reaction in the body. Benzidine and lueco-malachite green in the presence of peroxidase will turn a characteristic color indicating the possible presence of blood. Benzidine is prepared by mixing 0.25 grams of benzidine, 175 milliliters of ethanol, and 5 to 10 drops of glacial acetic acid together. Once mixed, this reagent should be stored in a dropper bottle in the refrigerator until used.[59] For conducting the test another dropper bottle containing hydrogen peroxide is necessary. The procedure for conducting the Benzidine Test in the field is as follows:

1. Lightly moisten a cotton swab with water.
2. Rub the swab over a small portion of the suspected stain.
3. Add two drops of the benzidine solution. (A blue color forming at this stage indicates a negative test.)
4. Add two drops of hydrogen peroxide to the swab.
5. A positive result is the rapid appearance of a blue color.[60]

The investigator should wear gloves and take necessary precautions when using benzidine, as it has been determined to be a cancer causing agent. Leuco-malachite green is prepared in two parts; a dry mixture and a wet mixture. The dry mixture contains 0.32 grams of sodium perborate, and 0.10 grams of leuco-malachite green. These two ingredients can be thoroughly mixed and stored at room temperature for up to a year. The wet mixture consists of 6.6 milliliters of glacial acetic acid diluted with 3.3 milliliters of water.[61] Just prior to use, the dry chemicals are dissolved in the wet mixture. The application of the leuco-malachite green test in the field should consist of the following procedure:

1. Lightly moisten a cotton swab with water.
2. Lightly rub the swab on a small portion of the suspected stain.
3. A few drops of the leuco-malachite green reagent is then added to the swab.
4. A positive result is the appearance of a deep green color.[62]

An easier method by far for conducting a presumptive blood identification test in the field is through the use of Hemastix.® Hemastix is the trade name for a product manufactured by Miles Laboratories. This product is a small

plastic strip with a chemically treated piece of dry absorbent material at one end. This test is also based on the peroxidaselike activity of the heme group.[63] The test is conducted by lightly moistening the absorbent material with water and then gently rubbing it on a small area of the questioned stain. A blue color is a positive result indicating the possible presence of blood. Hemastixs are sold in bottles containing fifty strips and represent an easy method for conducting presumptive blood identification tests in the field.

GENERAL CHARACTERISTICS OF HUMAN SEMEN

Semen is a complex fluid produced by the male reproductive system. Semen is usually white to yellowish in color and is ejaculated, or expelled, from the penis.

The average human ejaculate is approximately 3.5 milliliters, or about one teaspoonful, and contains approximately 350 million spermatozoa.[64] Semen is composed of two fractions: the liquid portion, called the seminal plasma, and the cellular portion, the spermatozoa.

The liquid portion, seminal plasma, is a thick complex mixture of secretions from the male reproductive organs and serves as the transport medium for the spermatozoa cells. One of the chemical components of semen is called flavin. This chemical is responsible for the somewhat yellowish color of semen, which causes the semen to fluoresce, or glow, in the presence of ultraviolet light. This characteristic can serve as an important means of locating semen at crime scenes.[65]

The cellular portion of semen is the spermatozoa. Spermatozoa are the male reproductive cells and contain one half of the genetic information necessary for the formation of the fetus. Spermatozoa are approximately 1/500 of an inch in length and are structurally composed of four parts.[66] The head of the cell contains the genetic information and is usually teardrop shaped. The shape of the head is the most striking difference among the sperm cells of the various animal species. The neck joins the head and the midpiece of the cell. The midpiece produces the energy necessary for the movement of the cell, and the tail is necessary for the mobility. The tail of the spermatozoa accounts for nine-tenths of the cell's overall length. The identification of one intact sperm cell, by the crime laboratory on a submitted stain, is positive proof of the presence of semen.[67] Figure 2.2 is a schematic drawing of a human spermatozoa cell.

THE IDENTIFICATION OF SEMINAL STAINS AT THE CRIME SCENE

Although blood is the most common form of serological evidence found at a crime scene, semen runs a close second because of the high incidence of sex

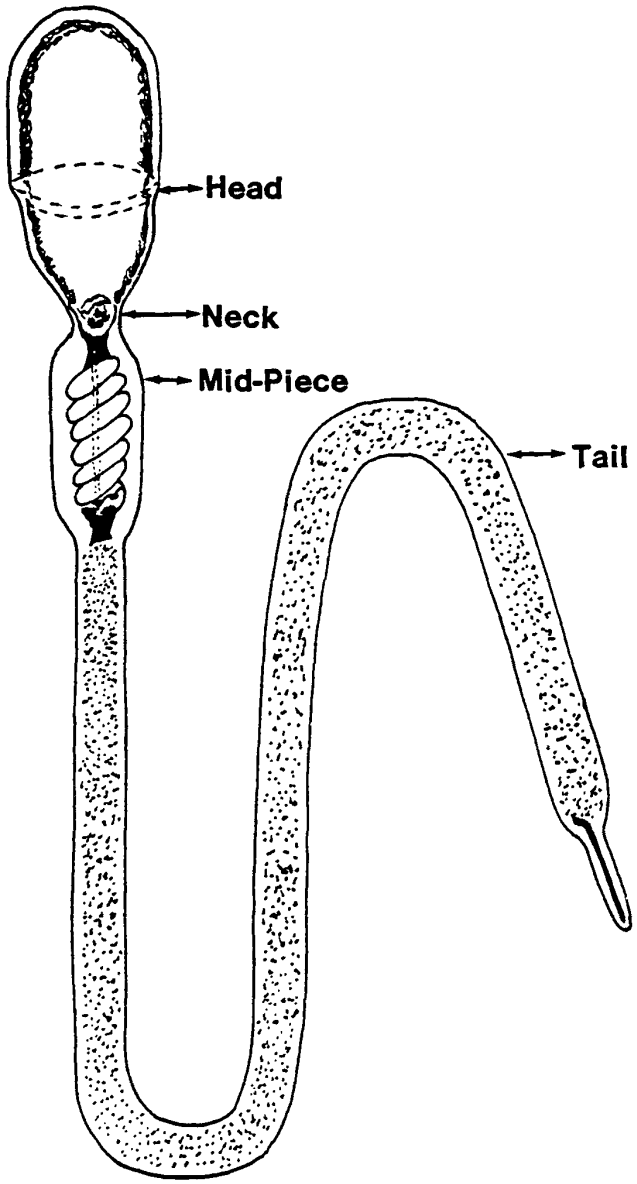


Figure 2.2. A diagramatic representation of a human spermatozoa cell.

crimes.[68] The search for seminal stains is often very difficult for the investigator, as they are hard to recognize.

The use of ultraviolet light to detect the presence of seminal stains can be useful, due to the strong bluish-white fluorescence of the stains. However, the

use of ultraviolet light has been severely impaired due to interference from current detergent brighteners.[69] Sheets, shirts, underwear, and other similar materials can carry highly fluorescent brighteners that are derived from the detergents used to launder them. Even if the investigator obtains the proper color of fluorescence from a stain, it is not proof, but only an indication that semen may be present.

In cases of rape or other sex crimes, the area of contact should be examined for the presence of seminal stains. Seminal stains are usually stiff and crusty feeling and can sometimes be located by feeling areas with one's fingers.[70]

Although seminal stains are usually found at the scene of rape and other sex crimes, they may also be present at burglaries, homicides, arsons, and other types of crimes.[71] For this reason every item suspected of possessing a seminal stain should be collected and submitted to the crime laboratory for analysis.

GENERAL CHARACTERISTICS OF SALIVA

Saliva is a thin, colorless fluid secreted by various glands of the mouth. Saliva moistens and begins the chemical digestion of food. Saliva is composed of 99.4 percent water and 0.6 percent solid material.[72] An enzyme present in saliva, amylase, is useful for the forensic identification of it in the crime laboratory.

In approximately 80 percent of the human population, known as secretors, the ABO blood group can be determined from the analysis of an individual's saliva.[73] For this reason, the recognition and recovery of suspected saliva stains from the crime scene is important.

THE IDENTIFICATION OF SALIVA STAINS AT THE CRIME SCENE

Saliva stains may be located on various items present at crime scenes. The investigator should examine and collect such items as cigarette butts, drinking glasses, handkerchiefs, and pillow cases from rape scenes.[74] Any and all items suspected of possibly containing saliva stains should be collected and submitted to the crime laboratory for analysis.

When a case involving bite marks is encountered, an expert from the laboratory should be contacted. It is possible for the expert to obtain saliva from the bite mark. This saliva, as will be explained in Section II, can be analysed for identification and exclusion of possible suspects.

Chapter 3

THE INTERPRETATION OF BLOODSTAINS AT THE CRIME SCENE

INTRODUCTION

THE distribution, quantity, and physical appearance of blood at a crime scene can often provide valuable information concerning the events of the crime. As Paul Kirk states, "No other type of investigation of blood will yield so much useful information as an analysis of the blood distribution patterns."[5] For this reason the investigator should examine and record all bloodstains at the crime scene. A detailed interpretation of bloodstain patterns often requires the services of a specially trained expert. However, there are some general principles that can be used by the investigator to reconstruct the crime scene and gain valuable information concerning the crime.

INTERPRETATION OF BLOODSTAIN PATTERNS

The physical appearance of bloodstains, found at the crime scene, may be useful in reconstructing the events that produced them.[6]

Bloodstain patterns lend themselves to geometric interpretation, which can reveal their origin and the mechanism that produced them.[7] Extensive research on the interpretation of bloodstain patterns has been conducted by H. L. MacDonell, Director of the Laboratory of Forensic Science in Corning, New York. MacDonell is considered the leading expert in this field, and his work stands as the current standard of reference.[8] MacDonell's research has led to the development of techniques for interpreting bloodstain patterns. By applying these techniques it is possible to determine the height, direction of travel, angle of impact, and velocity of the liquid blood that formed the stain. These interpretations involve application of the principles of geometry and physics to the patterns observed. For this reason the interpretation of bloodstain patterns is best left to a forensic expert who is trained and qualified in this

area. As MacDonell has stated in his book *Blood: Flight Characteristics and Stain Patterns of Human Blood:*

> It is this author's opinion that before anyone is qualified to render expert testimony on the significance of bloodstain patterns, they must have conducted a variety of experiments under known conditions using human blood, preserved their results as standards for reference, and have made a detailed study of these standards.[79]

However, there are some general principles that can be used by the field investigator for interpreting bloodstain patterns observed at the crime scene. Blood that has left the body, due to bleeding, will conform to the laws of ballistics.[80] Ballistics is the part of physics that deals with the behavior of projectiles in motion. The drops of liquid blood that fall from a wound will be held together in a spherical shape by cohesive forces.[81] These cohesive forces result in a type of "skin" forming on the surface of the drop, which is known as surface tension. This surface tension holds the drop in a spherical shape and prevents it from breaking apart, or separating, while falling through the air.[82]

When liquid blood drops from a wound, it does so in a uniform manner.[83] As each drop is being formed, its volume continues to increase until its weight allows it to separate from the surrounding blood. The average volume of a drop of blood dripping from a wound has been determined, by MacDonell, to be 0.05 milliliters.[84]

It is often useful to determine the distance that a drop of blood fell in producing a given stain. As stated earlier, the surface tension of a drop of liquid blood holds it intact in a spherical shape while it is falling through the air. When a drop of blood strikes a surface, surface tension also causes it to resist rupturing, or breaking apart. For this reason, a drop of blood that falls freely through the air and strikes a smooth, flat, hard surface such as glass, will not burst but will form a circular shape. However, when a drop of blood strikes a porous or rough surface, the surface tension is ruptured, causing the drop to splatter.[85] A falling drop of blood can be compared to a water-filled balloon. The rubber of the balloon is similar to the surface tension of a drop of blood. If the balloon is dropped on a hard, smooth surface, the rubber will expand and the balloon will not rupture. However, if the balloon falls on a piece of sandpaper, the coarse grains of the sandpaper will rupture the rubber balloon causing the water to splatter. When the surface tension of a drop of blood is ruptured, the edges of the stain pattern will have a scalloped or serrated appearance. Contrary to the information presented in many books written on crime investigation, these scallops are produced as a result of the surface a drop of blood strikes rather than the distance it falls.[86] Figure 3.1 illustrates this principle. Photographs A, B, C, D, E, and F were taken of single drops of blood, each of which fell 12 inches at a 90-degree angle and struck (A) a textured floor tile, (B) a piece of hardwood oak floor, (C) a paper towel, (D) a smooth piece of glass, (E) a piece of unfinished oak, (F) a smooth piece of white cardboard. It is

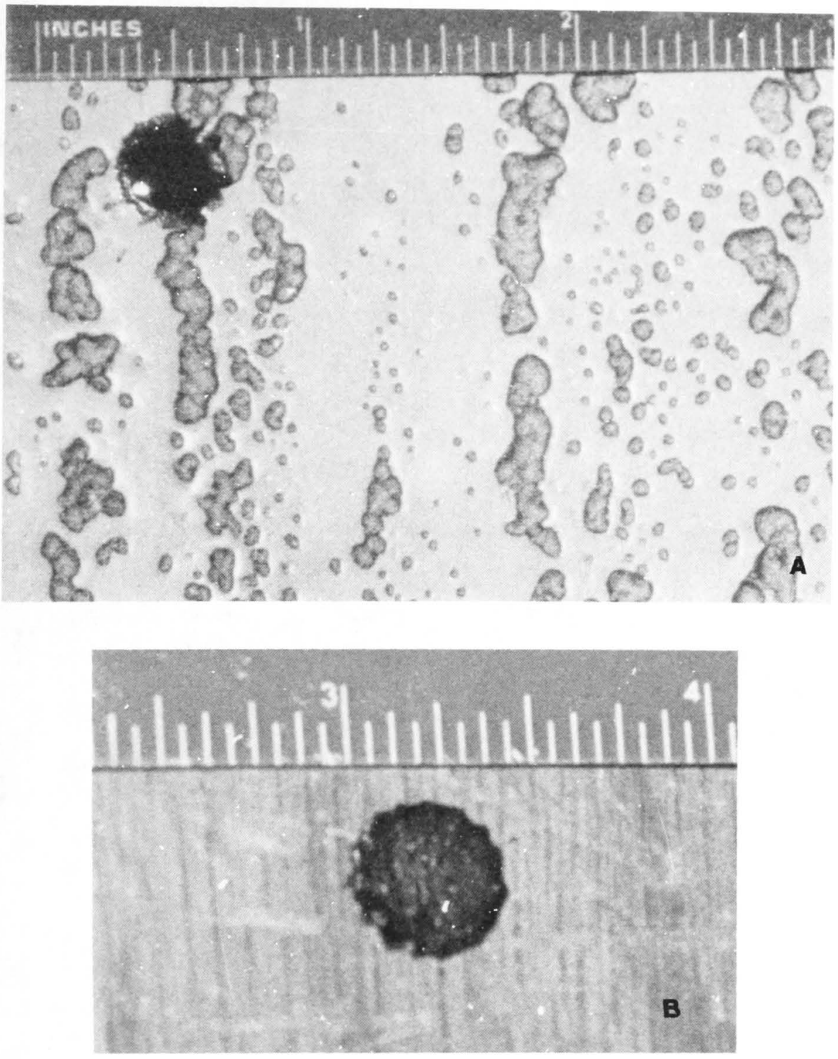


Figure 3.1. Photographs of a drop of blood that fell twelve inches when dropped from a 90-degree angle and struck **A.** A textured floor tile. **B.** Hardwood oak floor.

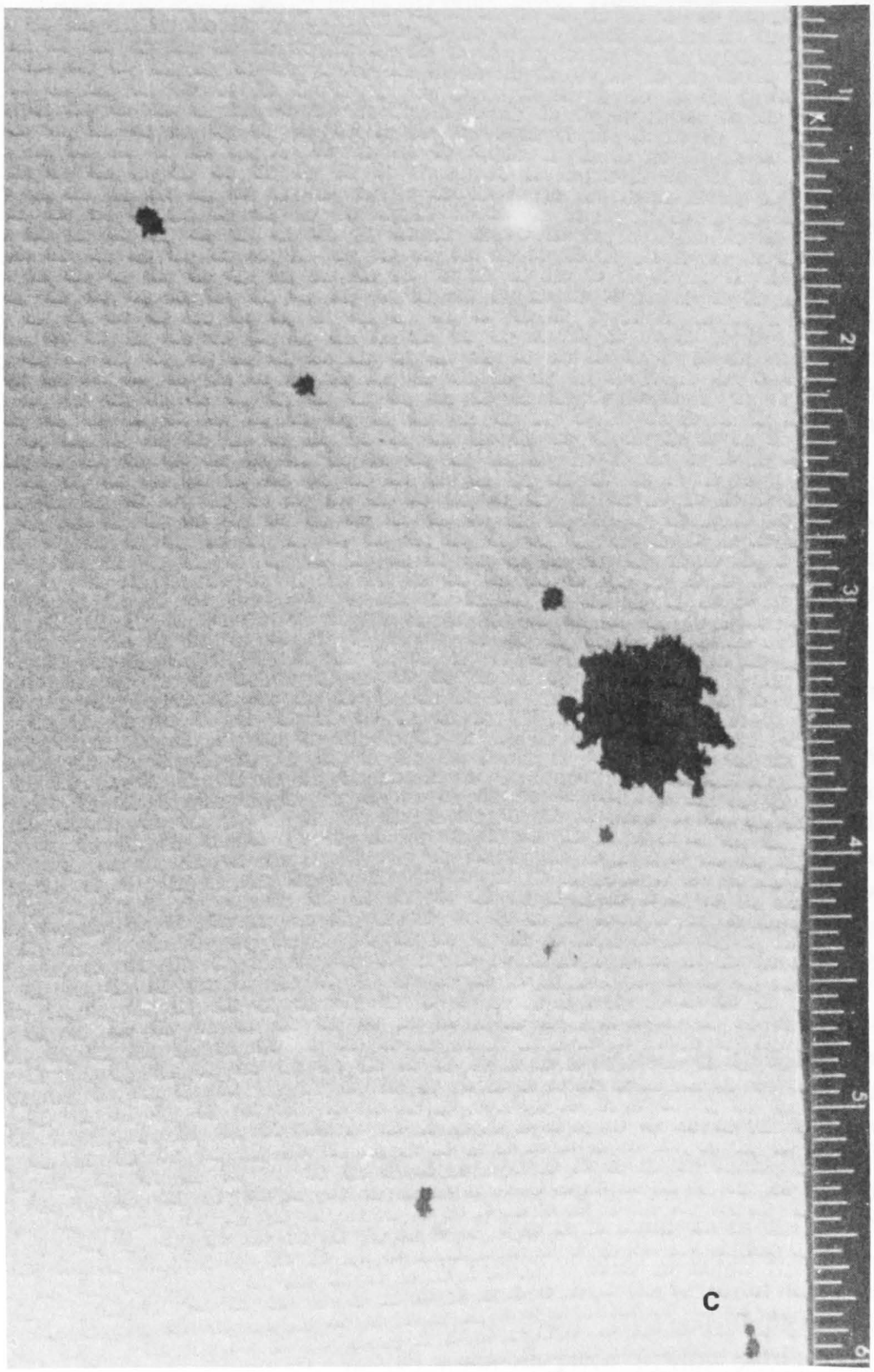


Figure 3.1. C. Paper towel.

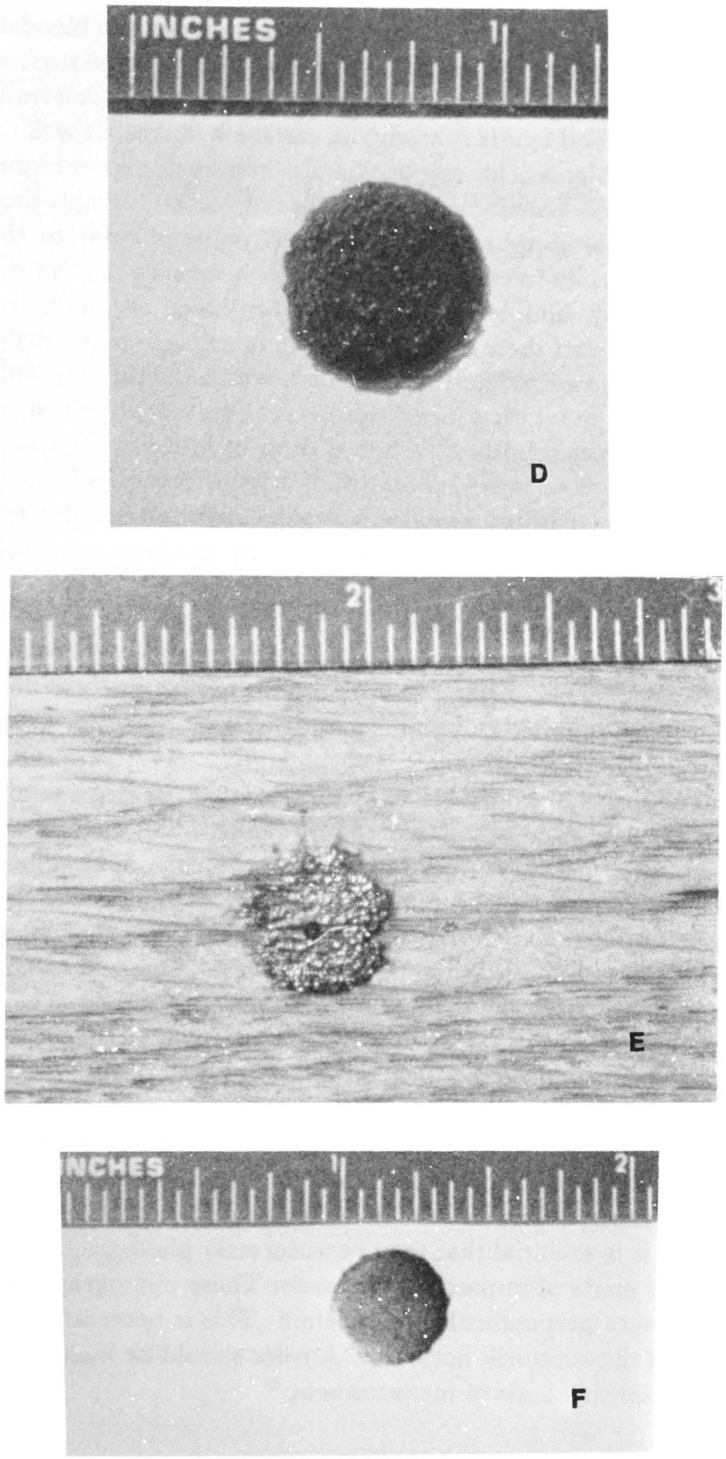


Figure 3.1. D. Smooth glass. **E.** Unfinished oak, furniture grade. **F.** Smooth white cardboard.

apparent from these photographs that the edge scallops of a bloodstain have little value in interpreting the distance the drop fell, unless the surface it strikes is considered. For these reasons the only accurate method of determining the distance a drop of blood falls is to study the surface it strikes, as well as the pattern produced by the blood. This can be done by a forensic expert in the laboratory. Therefore, it is necessary for the investigator to photograph the stain accurately. These photographs should be taken perpendicular to the stain and should include a ruler to establish a scale. It is necessary to submit these photographs along with samples of the surface on which the stain is found. The forensic scientist can then conduct a series of experiments on the surface in question and accurately determine the distance the blood drop fell.[87]

The direction in which a blood drop was traveling when it struck an object is not difficult to determine.[88] When a drop of blood strikes an object at an angle other than 90 degrees, a teardrop-shaped stain will be produced. The sharp point of this teardrop shape will face the direction the blood was traveling before striking the object.[89] When a drop of liquid blood strikes an object at a very sharp angle, a small droplet of blood will separate from the larger drop. This droplet will travel along the surface of the object, streaking it in a straight line. When this smaller droplet stops, a rounded end will be formed. In this type of bloodstain the pointed end represents the origin, not the direction of travel; the small rounded end points in the direction of travel.[90] Figure 3.2 illustrates this.

When a drop of blood falls at an angle close to 90 degrees, or almost straight down on a surface, a close examination of the edge scallops will reveal the direction of movement. The edge of the stain with the most edge scallops indicates the direction of travel.[91] Figure 3.3 illustrates this type of stain pattern.

Determinations can be made to indicate the origin of the blood producing a stain. These determinations require complex mathematical calculations of the angle of impact and should be done by an expert in the field. These determinations may be valuable in disproving an alibi offered by a suspect. For example, the bloodstains produced from shooting an individual who was sitting will be different from those produced from shooting the same individual standing. Some rough interpretations can be made by the investigator in the field. Generally, the longer and narrower the stain, the smaller the angle of impact.[92] Figure 3.4 illustrates examples of this. Whenever these bloodstain pattern types are located, it is essential that they be accurately photographed so determinations of their angle of impact can be made. These photographs must be taken with the camera perpendicular to the stain. This is necessary so distortions of the angles of the stain will not occur. A ruler should be included in the photograph to establish a scale of measurement.[93]

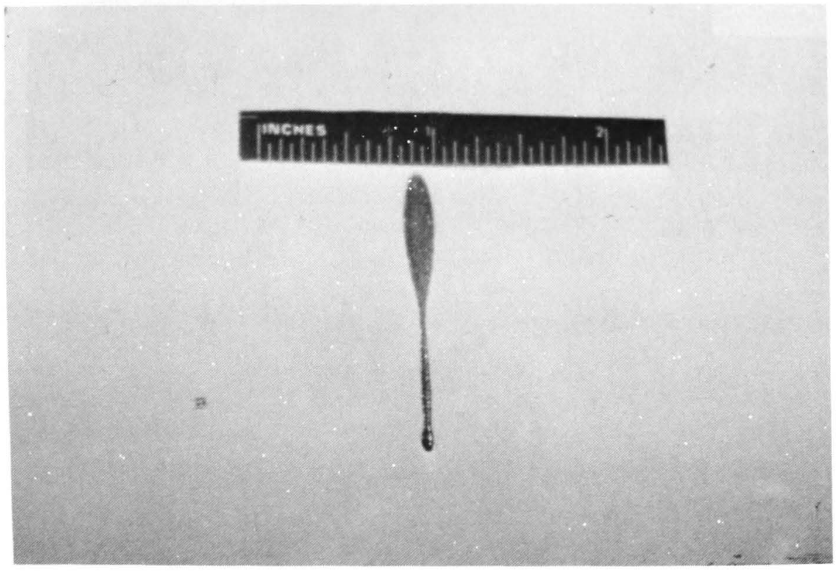


Figure 3.2. A photograph of a drop of blood that fell twelve inches and struck a piece of smooth, white cardboard at a sharp angle. The large, pointed end indicates the origin, and the small rounded end points in the direction of travel.

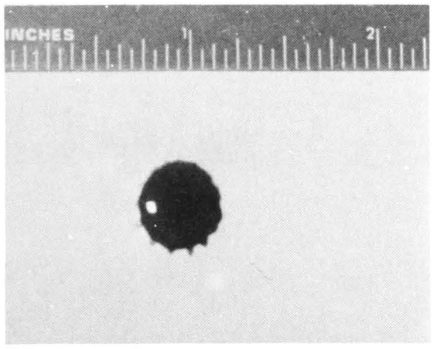


Figure 3.3. Photograph of a drop of blood that fell twelve inches when dropped from an angle of slightly less than 90 degrees. The preponderance of edge scallops on the lower edge indicate the direction of travel.

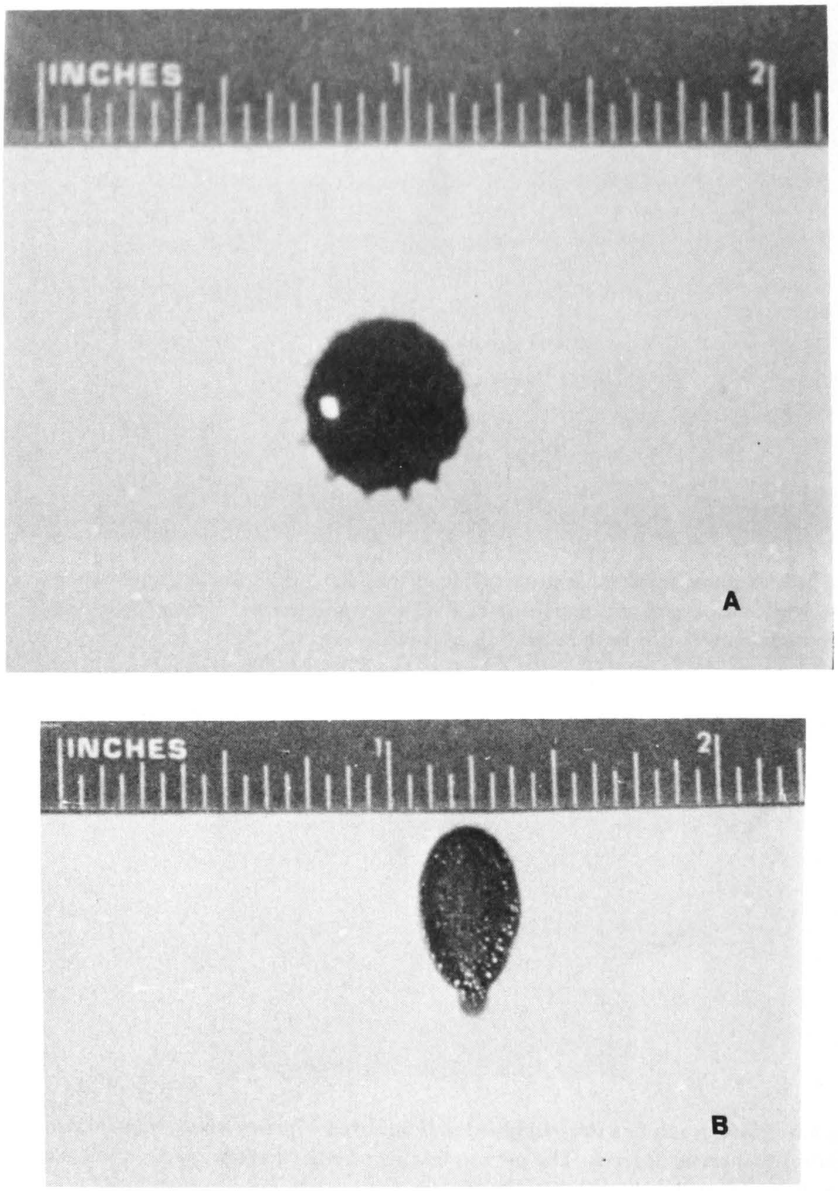
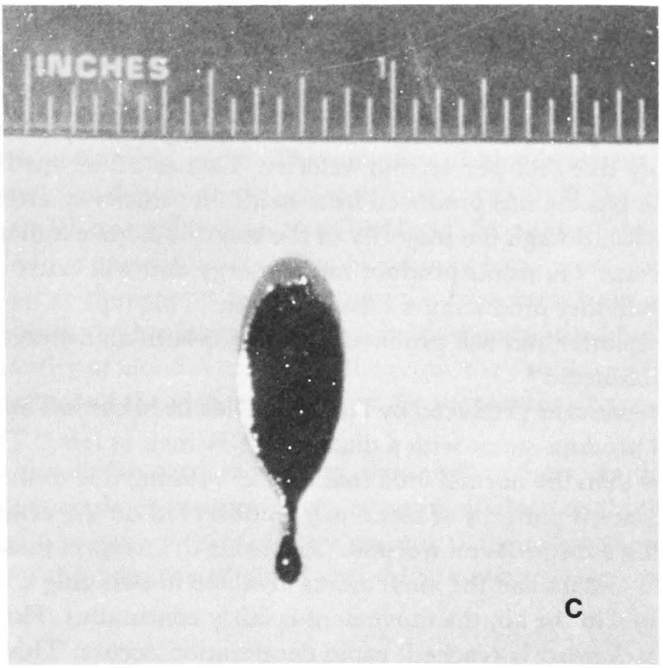
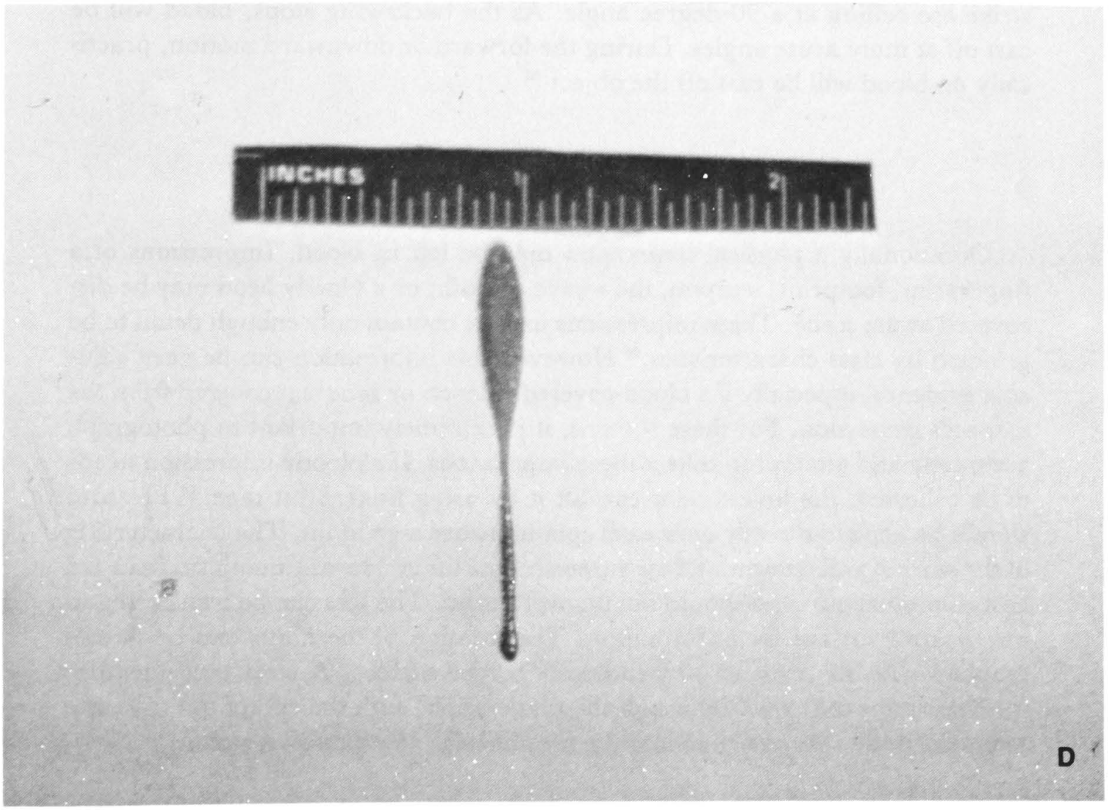


Figure 3.4 Photographs of a drop of blood that fell approximately twelve inches and struck a piece of smooth, white cardboard at approximately **(A)** an 80 degree angle, **(B)** a 60 degree angle, **(C)** a 40 degree angle, and **(D)** a 10 degree angle.


INCHES
1
C


INCHES
1
2
D

Bloodstains produced from a beating or a gunshot will exhibit a characteristic pattern. When energy is applied to a drop of blood, this energy will break the surface tension and result in a fine splattering of the blood.[94] MacDonell has determined that splatters resulting from most beatings usually occur at approximately twenty-five feet per second velocity. This is called medium velocity splatter.[95] The bloodstains produced from medium velocity splatter will appear quite fine in size, though the majority of the stains will have a diameter over 1 millimeter in size. Gunshots produce more energy and will cause a blood drop to further subdivide, producing a mist-like spray. This type of impact is called high velocity splatter and will produce bloodstains with an average diameter of less than 1 millimeter.[96]

Bloodstain patterns produced by blood that has been cast off or projected by an object will produce stains with a diameter of ¼ inch or less.[97] The drops will usually be less than the normal 0.05 milliliter in volume, due to the force of this projection. Cast-off patterns of blood can be observed on the ceiling as the result of swinging a blood-laden weapon. To be able to interpret these patterns, it is necessary to understand the movements involved in swinging a weapon. As a weapon is raised in the air, the movement is fairly continuous. However, as the limit of this backswing is reached, rapid deceleration occurs. This deceleration will cause blood to be flung or cast off the object. These drops of blood will strike the ceiling at a 90-degree angle. As the backswing stops, blood will be cast off at more acute angles. During the forward or downward motion, practically no blood will be cast off the object.[98]

BLOODY IMPRESSIONS

Occasionally a physical impression may be left in blood. Impressions of a fingerprint, footprint, weapon, the weave of cloth, or a bloody head may be discovered at the scene. These impressions usually contain only enough detail to be grouped by class characteristics.[99] However, this information can be very valuable evidence, especially if a blood-covered weapon or shoe is recovered from the suspect's possession. For these reasons, it is extremely important to photograph accurately and attempt to collect these impressions. If a bloody impression needs to be collected, the investigator can lift it by using fingerprint tape.[100] Pressure should be applied directly over each spot to insure a good lift. The characteristic of the surface will determine how successful the lift is. Hard, smooth surfaces are best, but other surfaces should not be overlooked. The lifts can be transferred to any neutral surface for examination. The location of these lifts can be photographed with the camera perpendicular to the surface. A steel tape measure should extend completely through the photograph, with the end of the tape at a reference point, but not touching the lift material, to establish a scale.

ESTIMATING THE QUANTITY OF BLOOD AT THE SCENE

Other useful information can be obtained from estimating the quantity of blood present at a crime scene. The quantity of blood present should bear a reasonable relationship to the injuries of the victim.[101] If the quantity is too large or too small, an explanation must be found. In cases where it appears that too much blood is present, some of the blood may have come from the suspect. It is important in these cases to collect samples from each stain as the serological analysis by the crime laboratory may yield important information.

If the quantity of blood is too small, the crime may have actually occurred at another location and the victim moved, or the suspect may have cleaned up the area.

Caution should be used in making these estimations, as the usual error, made by most people, is to estimate the quantity of blood too high. For this reason, it is useful to have several officers present at the scene to make individual estimations of the quantity of blood and then to compare and confirm the results.[102]

Chapter 4

RECORDING OF SEROLOGICAL EVIDENCE

INTRODUCTION

AS previously discussed, every item of physical evidence must be noted and photographed before it is collected. In the words of Hans Gross, "Never alter the position, or pick up, or even touch any object before it has been minutely described in an official note and a photograph taken."[103] This step is necessary to show the original position and nature of each item of evidence and the conditions of the crime scene. This chapter will present general techniques of note taking, crime scene sketching, and photography with an emphasis on the recording of serological evidence.

FIELD NOTES

The field notes written by an investigator serve as his record of the crime scene.[104] These notes are valuable in preparing written reports concerning the investigation and in refreshing the investigator's memory prior to testifying in court.[105] For these reasons the field notes should include enough detail so that they are still meaningful months after they are written. The field notes should begin with the start of the investigation and end with its conclusion. Every observation made, or fact discovered, should be recorded. Every investigation is unique, although there are some essential items that must be included in an investigator's field notes:

1. The date, time, location, weather conditions, light conditions, and the names of all persons present at the scene.
2. A detailed description of any victims.
3. The location, type, and size of any wound the victim has.
4. A general description of the crime scene.
5. Type of film, kind of camera, and lens used in photography.
6. An accurate description of every item of evidence found. This should

include the time and name of the person finding the item as well as the location. Accurate measurements of the position of the items should be included.

7. A record of the chain of custody of the item should be recorded. The procedures used in packaging and marking the item in custody should also be recorded.[106]

Every blood stain discovered should also be recorded in field notes including such information as—

1. the form of the bloodstain, liquid or dry;
2. the color of the stain;
3. the size of the stain;
4. the location of the stain; accurate measurements are necessary;
5. any estimates of the possible origin of the stain. This may include such things as estimation of angle of impact, direction of travel, and the mechanism by which it was caused.[107]

CRIME SCENE SKETCHES

Crime scene sketches are essential to provide a thorough understanding of the circumstances of the crime. Properly prepared sketches can—

1. supplement the crime scene photographs and field notes giving them the proper perspective, and show details not captured on film;
2. indicate the perimeter of the crime scene and locations of all items;
3. refresh the investigator's memory for later court testimony.[108]

All crime scene sketches should include the following information:

1. Agency and case number.
2. Date and time.
3. Entire perimeter of the scene. Detailed sketches can be made to show locations of bloodstains in particular rooms.
4. Location of the scene.
5. Locations of nonmovable objects such as walls, windows and built-in cabinets. These can be used as reference points for measurements.
6. Locations of all items of evidence by item number.
7. Dimensions of the scene and relationships between items of evidence.
8. The names of all persons involved in making measurements and preparing the sketch.[109]

Only facts should be included in the sketch. Conclusions such as labeling a gun the "murder weapon" may make the sketch inadmissible in court.[110]

Different types of sketches can be used, each type having a different purpose.[111] Several different types can be utilized to show different aspects of the scene. The projection drawing is one in which all places and objects are drawn as they would be seen from above. A variation of this is the cross-projection sketch. The cross-projection sketch is drawn with the walls folded out flat in the

same plane as the floor. This type of sketch is useful to illustrate the relationships between objects on the walls and floor of a scene. For this reason the cross-projection sketch is excellent for showing the relationship between bloodstains on the floor and the walls of a crime scene. Figure 4.1 illustrates this type of sketch.

A schematic drawing illustrates an event. The most common use for this type of sketch is to illustrate the events of a traffic accident. However, this type may be constructed by an expert to illustrate the events that could have caused a particular bloodstain pattern. This is illustrated in Figure 4.2.

A detailed drawing is used to enlarge a portion of the projection drawing. This is necessary when the scale of the projection drawing does not permit adequate illustration of some details. This type of sketch may be used to show the detail of bloodstain patterns at the scene.

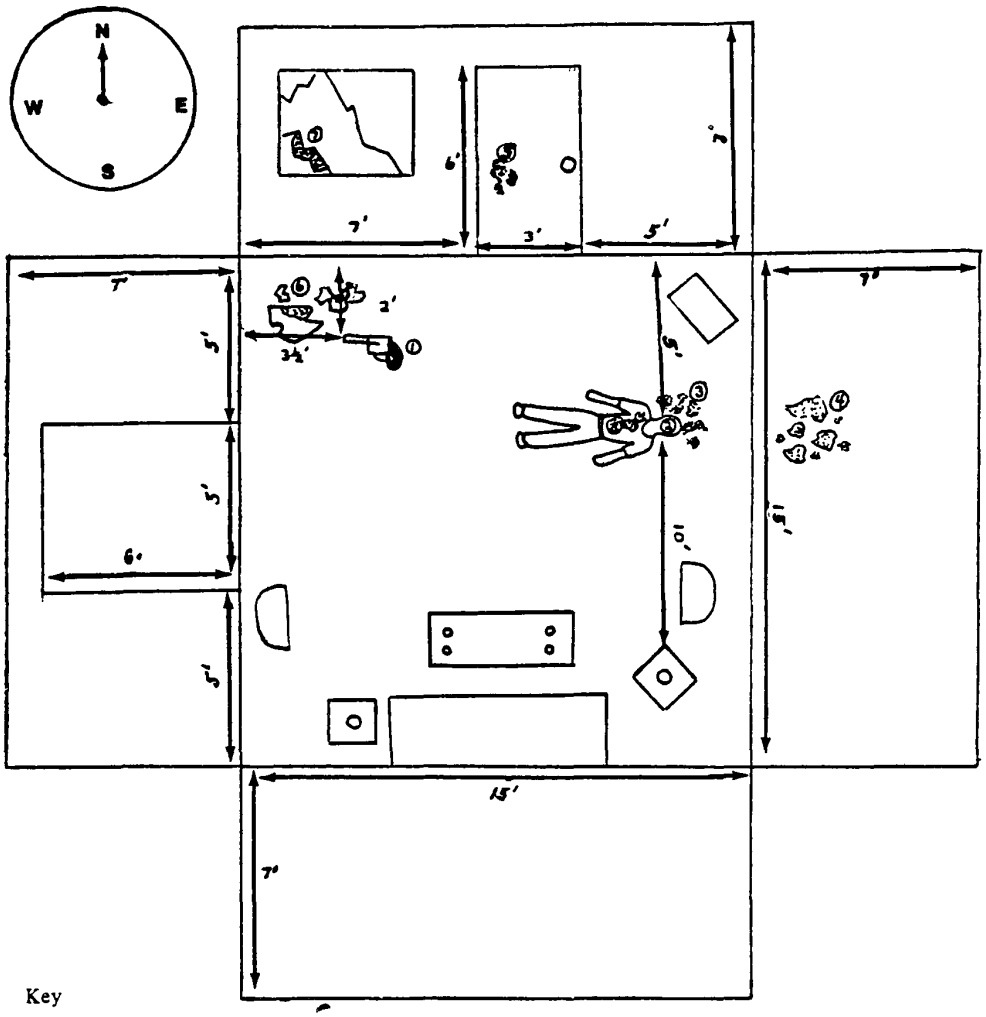
In constructing a crime scene sketch all measurements must be made as accurately and uniformly as possible. For example, some items should not be indicated in feet and inches, while others are indicated by the number of paces from a wall.[112] The most accurate and accepted method for measuring crime scenes is the use of a steel tape measure.[113] A rough sketch at the scene may not be drawn to scale, but all measurements must be included so that a scale can be established at a later time.

The locations of objects in a sketch must be shown by accurate measurements. There are two methods of measuring these locations; the coordinate method and the triangulation method. The coordinate method indicates the location of objects by distance measurements from at least two reference points (immovable objects such as walls, corners of buildings, or streets). These measurements must be made perpendicular, or 90 degrees from the reference point. This method is illustrated in Figure 4.3. If a reference point is unavailable, an imaginary straight line between two immovable objects can be drawn and used as a base line for measuring.[114]

Another method of measuring, which can be used in outdoor crime scenes, is the triangulation method. This method is useful when there are no objects such as buildings or roads to use as reference points. In this method, two or more reference points, such as trees, are selected. These points should be widely separated. The item is then located by drawing straight lines from the reference points, and carefully measuring the distances.[115] This method is illustrated in Figure 4.4.

Once the item has been located in a drawing, it is assigned an item number. This number should correspond to the number that is marked on the item as it is collected. This will preserve the identification of the item and eliminate the need for extensive writing on the sketch.[116]

A legend or key should be included in every sketch. This legend explains what each numbered item is and gives the location measurements of the item.



Key

1. Gun
2. Victim
3. Pool of Blood
4. Blood on East Wall
5. Blood Stain on North Door
6. Broken Glass on Floor
7. Bloodstains on Broken Glass
8. Blood on Chest

Scale – 1" = 1' 6"

Figure 4.1. An example of a cross projection crime scene sketch.

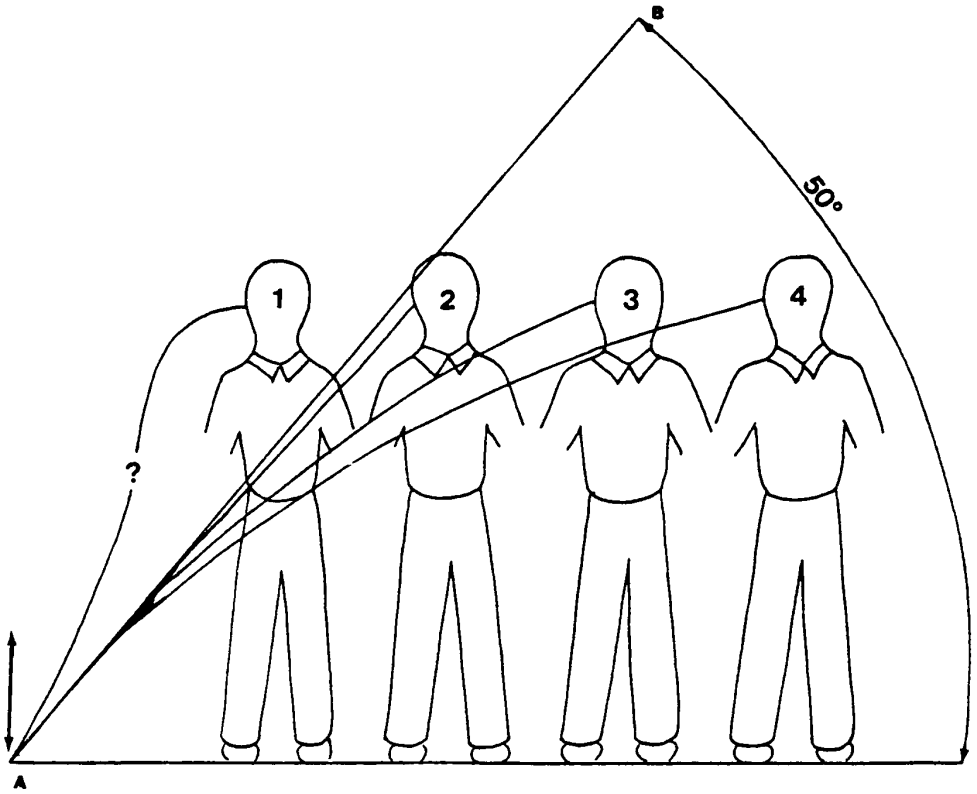


Figure 4.2. An example of a schematic crime scene sketch. Positions 2, 3, and 4 could have produced the blood stain on the floor. Position 1 is incapable of producing the blood stain. (Adapted from *Flight Characteristics and Stain Patterns of Human Blood,* by Herbert L. MacDonell.)

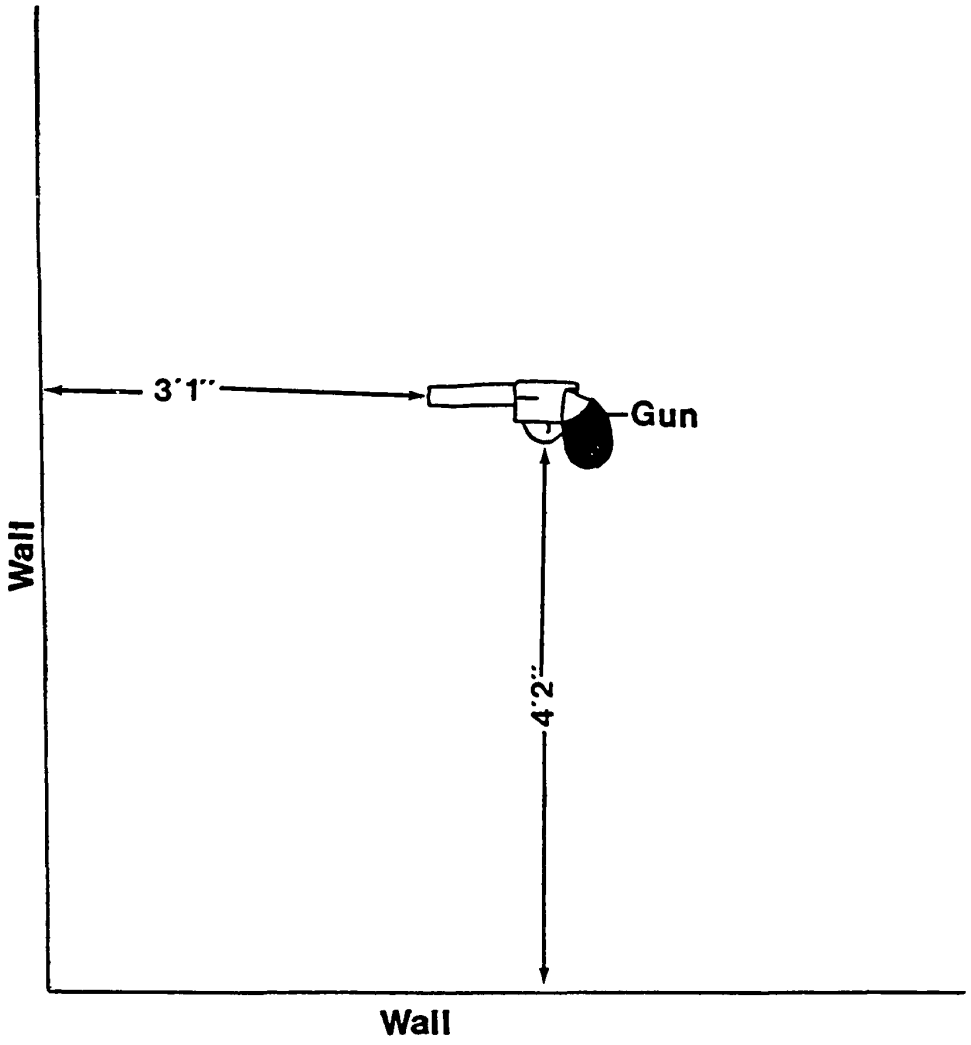


Figure 4.3. The Coordinate Method. The location of objects on a crime scene sketch is determined by measuring perpendicular to two reference points such as walls.

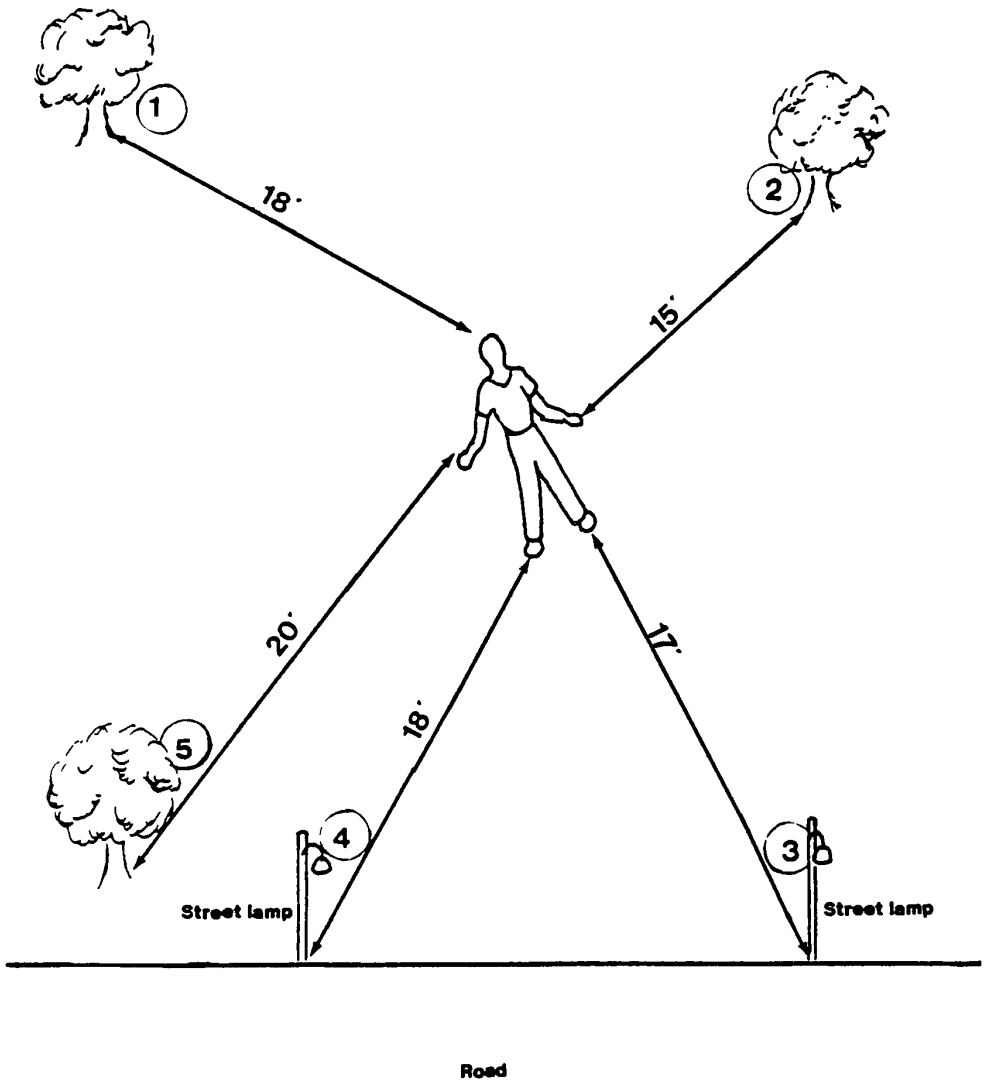


Figure 4.4. The triangulation method. Two or more separated objects (such as trees) are used as reference points for measuring.

For example a legend may contain "#4, 38 caliber, Smith & Wesson revolver, serial number 0001. Located 1'6" from south wall and 3'7" from west wall, with blood on the grips."

It is suggested that a police agency adopt standard symbols for depicting items commonly found at a crime scene.[117] One such set of standard symbols, recommended by Paul Weston and Kenneth Wells, is illustrated in Figure 4.5.

CRIME SCENE PHOTOGRAPHY

The photographs of a crime scene serve two purposes:

1. Provides the investigator with a permanent physical record of the scene and the items of evidence.
2. Allows the court and jury to obtain an accurate understanding of the situation as it was at the time the photographs were taken.[118]

Photographs of a crime scene often allow for the reconstruction of the events of the crime. Accurate photographs of the items of evidence are also often helpful to the forensic scientist in determining the types of analyses appropriate and as an aid in interpreting the results of the analysis.

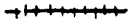
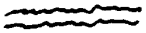
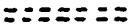
Photographs are admissible in court as an aid to testimony. It is often difficult for a group of individuals to obtain an accurate understanding of an event through verbal information only. Individual differences, past experiences, intelligence, and social and cultural differences all affect the interpretation of verbal information.[119] For these reasons photographic evidence is admissible as demonstrative evidence.[120] This simply means that photographs are admissible as an aid to verbal testimony. The photographs cannot serve as evidence by themselves, as someone must testify to their accuracy.[121] There are three requirements for the admissibility of photographs in court:

1. The object of the photograph must be relevant to the case. The photograph must be instructive or stress a particular point.
2. The photography must not appeal to the emotions or tend to prejudice the jury.
3. The photograph must be a true and accurate representation of the situation of the item.[122]

Photographs of a crime scene should include three different views—

1. general view should be taken with the camera at eye level so as to give the same perspective that a witness would have of the scene;
2. 10 to 20 feet from the object so as to show its relationship to other objects at the scene;
3. close up view of each item of evidence.[123]

In addition to these general photographs, others should be taken of—

Distance Measurement Line	
Items of Evidence	1. 2. 3 Etc.
Light Pole	
Traffic Signal	
Gun	
Knife	
Road or Driveway	
Fence	
Railroad	
Tree	
Hedge, Low Shrubbery	
Footpath Walkway	
Vehicle	 Front
Bicycle or Motorcycle	 Front
Camera Position	
Blood	
Body Victim	
Chair	
Sofa, Couch, Day Bed	
Table – Coffee or Large	
Table – Small	
Table – Round	
Lamp	

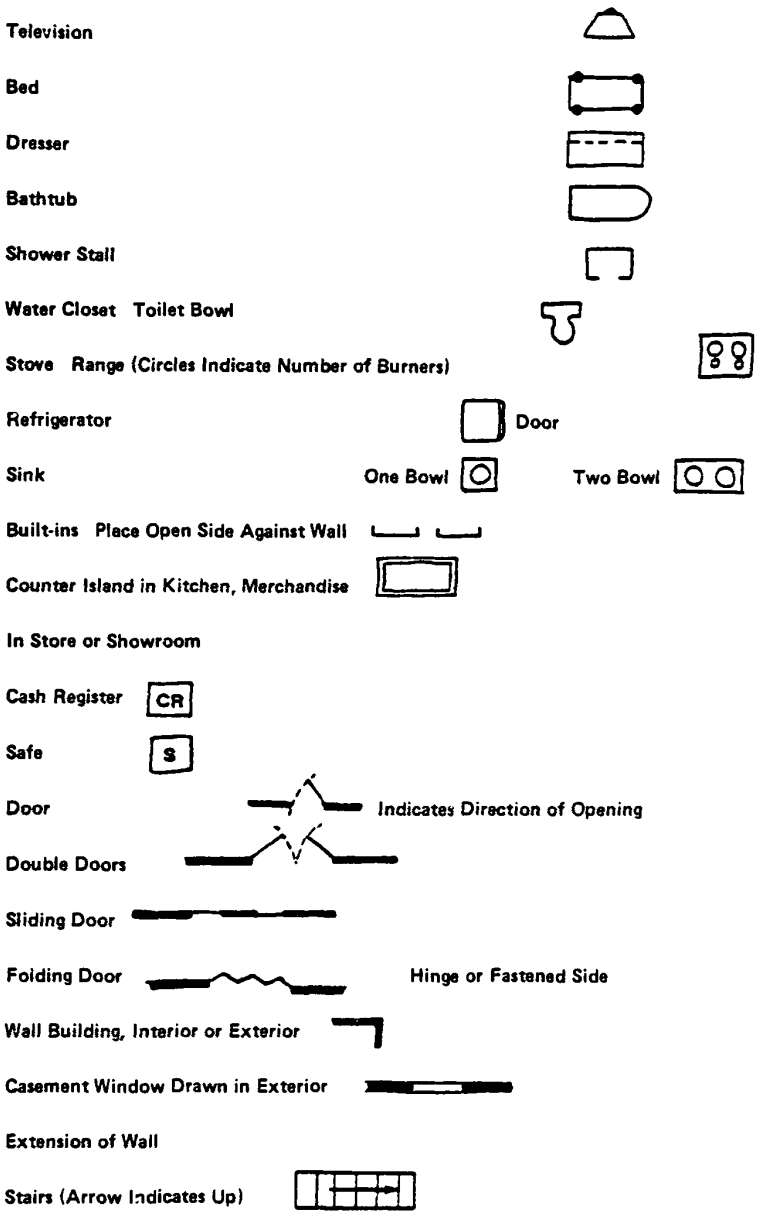


Figure 4.5. Standard symbols such as these may be used in making crime scene sketches. (Adopted from *Criminal Investigation,* by Paul Weston and Kenneth Wells.)

1. objects that establish the facts of the crime (the corpus delecti);
2. evidence that shows the manner in which the crime was committed (the modus operandi);
3. any objects that could possibly provide a clue to the identity of the suspect;
4. anything that shows the events of the crime, bloodstains, blood splashes, weapons, and signs of struggle;
5. fingerprints.[124]

Photographs of bloodstains should include a ruler to establish a scale of measurement. However, the defense may object to these photographs, claiming they are not true and accurate representations because of the ruler. For this reason it is necessary to take two photographs of each stain. One should be taken without the ruler, and one with the ruler.[125]

Detailed photographs of all bloodstains should be taken. These photographs should include a close-up of the stain as well as photographs showing the relationship between the stain and other objects. Photographs of bloodstains should be taken with the camera perpendicular to the stain. This is necessary so the size and shape of the stain is not distorted.[126]

Generally a camera lens with a focal length of approximately 50mm tends to produce photographs closely resembling those seen by the human eye. A wide angle lens (focal length of about 32mm) will exaggerate the distance between the camera and the object. A telephoto lens (focal length of 85mm or more) will reduce the apparent distance between the camera and the object.[127]

Chapter 5

THE COLLECTION AND PACKAGING OF SEROLOGICAL EVIDENCE

INTRODUCTION

AFTER serological evidence is noted and accurately photographed, it should be sent immediately to the crime laboratory for analysis. The techniques used by the investigator in collecting and packaging this evidence are crucial in preserving the evidence for forensic analysis. At a crime scene blood may be discovered on a wide variety of surfaces and objects. For this reason no single rule can be given for the handling of the evidence. However, there are some general guidelines that can be followed in collecting and packaging serological evidence. This chapter will present the legal and scientific requirements of evidence collection as well as some general techniques that can be used to collect blood and semen.

LEGAL AND SCIENTIFIC REQUIREMENTS

For an item of evidence to be admissible in court the investigator must be able to—

1. identify it;
2. describe the exact location and time of collection;
3. prove the chain of custody;
4. describe any changes that may have occurred between the time of collection and the introduction of the item in court.[128]

For these reasons each item collected must be marked for identification, located on a crime scene sketch, and recorded in field notes. It is desirable to keep the chain of custody short. For this reason two officers should collect all the evidence at the scene. If both officers sign the report and initial the evidence, either may testify in court. The reason for having two officers mark each item of evidence is illustrated in the following example. If one officer

collects all the evidence at twenty-five crime scenes in a one year period and is then killed, no one can testify to the evidence collected. However, if two officers collect the evidence at these crime scenes, one will still be able to testify to it.

Each item collected should be placed in an evidence bag, sealed, and marked with an item number, the officer's initials, type of crime, case number, and date. A property sheet is normally filled out listing the crime, case number, description of the items collected, submitting officers, and chain of custody. The item numbers on this property sheet should coincide with the item numbers on the crime scene sketch and other reports of the crime. This property sheet is attached to the evidence bag when submitted to the laboratory. These steps are essential to allow the evidence to be admissible in court.

The scientific requirements are basically that the evidence be protected from any contamination or modifications.[129] Serological evidence is subject to decay, and care must be used in packaging and collecting it. Some general rules regarding this type of evidence are the following:

1. The most important rule to be remembered is *never package serological evidence in plastic bags.* Plastic bags are air tight and any moisture will cause the evidence to decay, completely destroying it.
2. When a portion of an article containing blood is submitted, always include an unstained portion of the same article to be used as a control.
3. Prevent cross-contamination of blood stains. This requires that bloodstained articles be packaged separately.
4. When dried bloodstains are scraped up with a razor blade always use a clean razor blade and include it in the package.
5. Always use clean containers for packaging.
6. Wear gloves. This will guard against perspiration from the officer being placed on the article, which could interfere significantly with the analysis.

COLLECTION OF BLOOD EVIDENCE

By far the best method of preserving and collecting bloodstains is to take possession of the object on which it is found.[130] This is often possible when bloodstains are located on clothes, curtains, cushions, and small objects. When this occurs, the item is marked for identification and placed in a paper evidence bag to be sent to the laboratory. If the stains are still wet, they must be dried before packaging. Let the material air dry, never subjecting bloodstains to heat or sunlight, as this will destroy some of the potential for analysis.[131] Care is necessary to prevent wet bloodstains from touching other unstained portions of the article. This could lead to a transfer of the stain, making it impossible for the laboratory to determine the position of the body at the time of bleeding.[132] To prevent any transfer of bloodstains, clean paper should be placed under and on

top of the article. The article can then be gently rolled up and placed in a paper evidence bag.[133] To further protect against transfer of stains, each article of clothing should be packaged separately. If clothing contains cuts or tears that are bloody, do not fold it so that a cut is creased or stretched. This could destroy the ability of the forensic expert to match a weapon to the cut or tear.[134]

Bloodstains found on knives or guns may loosen and fall off when they are dry. This can be prevented by wrapping the bloodstained portion in clean paper and taping it securely before packaging.[135]

Liquid blood is often encountered at the crime scene. There are various methods that can be used to collect this liquid. The blood can be picked up with a clean medicine dropper and placed in a sterile test tube. Vacutainer® tubes can be purchased that contain EDTA, which is a type of preservative. This is the recommended preservative to use for liquid samples.[136] If these tubes are not available, the blood can be placed in a sterile test tube containing an equal amount of saline solution. This will also preserve the blood for analysis.[137] Liquid blood samples should be refrigerated or packed in ice during their transport to the crime laboratory. Never use dry ice, as this will freeze the blood, making analysis impossible.[138]

Small amounts of liquid blood can be soaked up using a small swatch of clean, 100 percent cotton cloth. Cotton bed sheets can be purchased and cut up for this purpose. Once the stain has been soaked up the swatch should be air dried before packaging.

Dried blood on walls, floors, and other objects can be scraped off with a clean razor blade. A separate clean blade should be used for every stain and packaged with the dried blood. The stain is scraped onto a piece of clean white paper, folded up, and packaged in a pillbox or other suitable container. A useful technique for scraping dried blood from walls is to tape a piece of clean white paper to the wall. The stain can then be scraped onto the paper.[139]

Whenever dried stains are cut out of an object, an unstained portion of the object should be included for a control.

Blood that has run into cracks of a floor can be difficult to collect. The surrounding floor may be removed and the blood scraped up, or a few threads can be pulled from a clean cotton swatch. These threads are moistened and rubbed in the crack to dissolve and absorb the blood.

An outdoor crime scene will usually require different collection techniques. Bloodstains located on the ground should be collected by digging up the dirt with a towel and packaged. Any worms should be removed, as they may eat the dried blood. Accurate measurements of the depth of the blood should also be recorded.[140] Bloodstains on the grass or other vegetation should be collected by cutting the vegetation and submitting it as dried blood evidence.

THE COLLECTION OF SEMINAL STAINS

All sexual assault scenes should be searched for the presence of seminal stains. Once possible stains are located, care is necessary in their collection. Seminal stains are very fragile when dry and can easily be destroyed.[141]

When a sexual assault occurs indoors, the exact location of the assault should be examined.[142] Bed sheets should be marked indicating the head and foot.[143] The reason for these markings is that sexual assaults are often unnatural acts and the location of seminal fluid may provide valuable information about the nature of the assault.[144]

Due to the fragile nature of seminal stains, sheets should never be folded or rolled up. Friction from careless folding may destroy the stain. This can be prevented by attaching a piece of clean cardboard to the stain before folding the sheet around the cardboard and placing it in an evidence bag.[145] Each item should be packaged in a separate paper bag to prevent transfer of the stain to an unstained portion.

Seminal stains located on wood floors should be carefully dislodged with a clean razor blade. This should be done very carefully so as not to destroy any spermatozoa cells present.[146]

The victim of a sexual assault should immediately be transported to a hospital. A doctor will use a rape kit at the hospital to gather the items of evidence from the victim. This kit should then be marked and transported to the crime laboratory. All of the victim's clothing should be collected, packaged separately, and sent to the laboratory for examination.

Tables 5.1 and 5.2 contain a summary of the major points concerning collection and packaging of blood and semen evidence.

Table 5 1

THE COLLECTION OF BLOOD EVIDENCE

ITEMS	*COLLECTION PROCEDURES*	*AMOUNT OF SAMPLE DESIRED*	*PRESERVATION*	*PACKAGING*	*INVESTIGATIVE VALUE*
Liquid Blood	Take up in a clean medicine dropper and place in sterile test tube containing EDTA, or an equal amount of saline.	All of sample	Refrigerate or pack in ice—*never use dry ice.*	Place in paper evidence bag and list: Date, item number, location at scene, collecting officers initials and case number. Seal evidence bag with evidence tape.	Possible determinations by crime lab include: 1. Species origin of blood (human or other animal) 2. ABO, MN & Rh blood group 3. Enzyme groups 4. Part of body blood is from 5. Sex of individual 6. Blood-Alcohol level 7. Toxicology-drug screen
	Absorb on piece of clean, 100% cotton cloth	All	Air dry the cloth before packaging.		
Dried Blood Stains	Collect entire object, if possible.	All	Be sure the sample is completely dry before packaging.	Place in clean, dry evidence bag and seal. Label item and bag with case number, item number, location of object, date, and initial.	1. Identify human blood 2. ABO blood group 3. Enzyme groups 4. Sex of individual
	Scrape up with clean razor blade. Place sample and razor blade in clean container.	All of Sample & a sample of an unstained portion of the surface for Control			
Bloody Clothes	Collect all bloodstained clothes from victim and suspect If it is necessary to cut clothing off, do not cut through any blood-stains or cuts.	All	Air dry. Roll up each item between 2 clean pieces of paper and place in paper evidence bag. Do not fold through any stains or cuts present.	Package each item in a separate clean paper evidence bag. Label bag with pertinent information, seal and initial.	1. Identify blood 2. Determine if human blood 3. ABO blood group 4. Blood enzyme systems 5. Identify sex of individual 6. May be able to match knife to any cuts in clothing.

Table 5.2

THE COLLECTION OF BLOOD EVIDENCE

SEMINAL	*COLLECTION PROCEDURES*	*AMOUNT OF SAMPLE DESIRED*	*PRESERVATION*	*PACKAGING*	*INVESTIGATIVE VALUE*
Seminal Stains on fabrics and clothes	Place clean cardboard over stain and gently fold up article.	All	Make sure the article is dry. Carefully fold.	Place each article in a separate clean paper evidence bag, seal, initial and include pertinent information.	1. Identify semen 2. May be able to Group (ABO & Enzyme) the stain
Dried Stains on floor	*Carefully* scrape up with clean razor blade. Place stain and razor blade in container.	All and sample of surface area	Carefully scrape.	Place stain in piece of folded clean white paper, include razor blade. Place in evidence bag. Seal and include pertinent information.	1. Identify semen 2. May be able to Group (ABO & Enzymes) the stain.

SECTION II
THE ANALYSIS OF SEROLOGICAL EVIDENCE

INTRODUCTION

FORENSIC science is "the study and practice of the application of science to the purposes of law."[147] The most useful division of forensic science to the criminal investigator is criminalistics, "the profession and scientific discipline which is directed towards the recognition, evaluation, identification, and individualization of physical evidence in law-science matters."[148] Forensic serology is a subfield of criminalistics and involves all examinations of blood and body fluids (often as dried stains) associated with civil or criminal matters.[149] There are three major aspects of forensic serology:

1. The identification of blood and body fluids especially in questioned stains.
2. Attempts to individualize the samples, once they have been identified, using serological and immunological techniques.
3. Blood grouping techniques in disputed paternity.[150]

The first two aspects are directly applicable to the investigation of a criminal offense and will be the subject of this section.

This section of the manual will not attempt to present detailed descriptions of the theories and techniques used in forensic serology, as this is unnecessary for the purposes of this manual. This section will give a general description of the commonly utilized techniques in order to give the investigator a general understanding of the value of serological evidence and the necessity for its proper collection and preservation.

Chapter 6

THE IDENTIFICATION OF SEROLOGICAL EVIDENCE

INTRODUCTION

SEROLOGICAL evidence is collected from a crime scene and should be submitted without delay to a criminalistics laboratory for analysis. The first step in the forensic analysis of serological evidence is to make a positive identification of the stain. Several techniques have been developed that can be used to positively identify blood, semen, and saliva stains. The technical aspects of these techniques are beyond the scope of this manual. Therefore, this part of the manual will present only general information concerning the most common identification techniques.

THE IDENTIFICATION OF BLOOD

Techniques for the positive identification of blood in questioned stains are based on the detection of the presence of heme. Hemoglobin is a chemical that is found in the red blood cells. As discussed in Section I, hemoglobin is composed of two parts; an iron containing pigment, heme, and a protein component, globin. Blood identification tests can be divided into two categories: the presumptive tests, which are nonspecific for blood, and the confirmatory, which are specific for blood.

The presumptive tests include the benzidine, leuco-malachite green, luminol, and the Hemastix.® These tests are called presumptive because a positive reaction is only an indication of the presence of blood, not definite proof. On the other hand, a negative reaction means that no blood is present and no further analysis is required. All of the presumptive tests are based on the peroxidaselike activity of the heme component on hemoglobin. Peroxidase is an enzyme that can speed up a chemical reaction in the body. The chemicals benzidine, leuco-malachite green, and the Hemastix in the presence of a

peroxidase, such as hemoglobin, will rapidly change to a characteristic color. The tests are very sensitive and can usually detect the presence of blood diluted with water in the ratio of 1:1,000,000.[151] These tests, however, are not specific, as other substances will also produce a positive reaction. Such substances can be divided into three general categories:

1. Chemical substances. Copper and nickel are the two most common chemicals that will produce a positive presumptive test result.
2. Plant sources. The following plant tissues will often produce positive results: apple, apricots, horseradish, potato, turnip, cabbage, and onion.
3. Animal sources. The following animal tissues will also produce a positive result: pus, bone marrow, spinal fluid, mucous, and saliva.[152]

All of the presumptive tests are easy to use and are applicable for use as field tests to indicate the presence of blood. These tests are also used in the crime laboratory as a preliminary screening method for submitted stains.

Confirmatory blood identification tests are specific for the heme component of hemoglobin. A positive confirmatory test result is taken as positive proof of the presence of blood in a questioned stain. There are many types of confirmatory blood identification tests, but the microcrystal tests are the most commonly used.[153] There are two microcrystal tests, the Teichmann and the Takayama. Neither of these two crystal tests is as sensitive as the presumptive tests and can generally only detect the presence of 0.001 milliliters of blood.[154] Confirmatory tests are also subject to interference from the surface material on which the stain is deposited. Bloodstains on wood and leather give the most interference and often result in a negative result.[155] For this reason a negative confirmatory test is not absolute proof that blood is not present in a stain.

Both the Teichmann and the Takayama confirmatory tests are based on the observation that heme, in the presence of certain chemicals, will form characteristic crystals that can be seen microscopically. Both of these tests are conducted using this procedure:

1. A few stained threads or a small portion of the dried stain are placed on a microscopic slide.
2. A few drops of either the Teichmann or the Takayama reagent are added to the slide.
3. The slide is heated, then observed microscopically for the presence of characteristic crystals.

THE IDENTIFICATION OF SEMEN

When the evidence of a sexual assault is submitted to a crime laboratory, the first step in the analysis is to identify the presence of semen. The positive identification of seminal fluid and spermatozoa cells from a stain can provide the

investigator with valuable information. These identifications can provide the investigator with information concerning the events of the crime, and if the crime is rape, the identifications of spermatozoa may tend to corroborate the victim's story. For this reason all items that may possess seminal fluid should be carefully collected and submitted to the criminalistics laboratory for analysis.

Semen is a complex fluid, white or yellowish-white in color, produced by the male reproductive system. Semen is composed of two fractions, the liquid portion seminal plasma, and the spermatozoa cells. The seminal plasma is a thick complex mixture of secretions from the male reproductive organs and serves as the transport medium for the spermatozoa cells. The spermatozoa cells are the male reproductive cells. The average male ejaculate contains approximately 350 million spermatozoa cells.[156] Considering the large amount of spermatozoa cells contained in the ejaculated seminal fluid, one would think that the chance of locating a single spermatozoa cell in a seminal stain is very good. However, spermatozoa cells are very brittle when dry and are easily destroyed. For this reason extreme care is necessary when collecting and packaging suspected seminal stains.

Both the seminal plasma and the spermatozoa cells can be identified from dried stains. Dried seminal stains are stiff and crusty to the touch and depending on this method for their identification is often unreliable, as other stains can also have this texture. The best method for locating the presence of seminal stains is the Acid Phosphatase Color Test.[157] Acid phosphatase is an enzyme secreted by the prostate gland of the male reproductive system. The concentration of acid phosphatase in seminal fluid is approximately 20 to 400 times greater than that found in other body fluids.[158] For this reason the detection of high levels of acid phosphatase is the method most commonly used to screen possible seminal stains. This test is relatively simple and consists of dropping a solution of sodium alpha-naphthylphosphate on a small portion of the dried stain. This solution is allowed to soak into the stain for approximately one minute. Then a drop of fast blue B dye is added. A blue-violet color appearing in thirty seconds is not considered a positive proof for the presence of semen, as other substances may produce positive results, but it is highly indicative of the presence of semen.[159]

A stain that gives a positive acid phosphatase reaction is then examined for the presence of spermatozoa. The stain is usually soaked in a small amount of saline to dissolve it. A few drops of this saline solution is then placed on a slide and observed microscopically for the presence of spermatozoa cells. Human spermatozoa cells are characteristic in appearance and differ greatly from spermatozoa cells of other species of animals.

The presence of semen in a questioned stain does not always mean that sperm cells will be present in the sample. There are many reasons why semen can lack sperm cells. The individual may have an abnormally low sperm count

(ogligospermia) or the complete absence of sperm in the seminal fluid (aspermia).[160] The absence of sperm may also be due to the techniques used for the collection of the stain. Finally, a doctor in the rape treatment center of the hospital may have missed any spermatozoa cells present when the vaginal swabs were taken. In cases such as these the forensic serologist must rely on the results of the acid phosphatase test in reporting the results of the analysis. Forensic serologists differ in their interpretations of the acid phosphatase test. Some believe that the presence of high levels of acid phosphatase is positive proof of the presence of seminal fluid, while others do not.[161] Opinion differences result from the fact that vaginal secretions also contain acid phosphatase. These vaginal secretions are normally lower in concentration than seminal fluid. This level varies from female to female, though, and no standard level can be determined. The recent discovery of a protein called P30 in seminal fluid may provide a positive means of identifying seminal fluid. Current research indicates that this protein is formed in the prostate gland and is present only in seminal fluid.[162] This protein can be identified by an immunological technique known as electrophoresis. Some of the forensic laboratories in this country are using the electrophoretic technique to identify the presence of P30, thereby identifying the presence of seminal fluid.

THE IDENTIFICATION OF SALIVA

Saliva stains are very difficult to identify positively due to the lack of sufficient amounts of detectable substances present.[163] However, there are chemical procedures that can identify amylase, an enzyme present in saliva. These tests are based on the ability of amylase to break down starch in foods. The tests are conducted by cutting a small portion of the suspected stain and placing it in a test tube. A starch solution is added to the test tube and is incubated, or heated. If amylase is present in the stain, the starch will be broken down. This is tested by the addition of iodine to the solution. No change in color is considered a positive result.

Saliva can be valuable evidence, because it is sometimes possible to determine an individual's ABO blood group from an analysis of his saliva. These determinations are due to the fact that approximately 80 percent of the human population are secretors. Secretors are individuals who secrete their ABO blood group substances in their saliva. Male secretors also secrete their ABO blood group substances in their seminal fluid. A forensic analysis of semen and saliva stain can, therefore, indicate the individual's ABO blood group. This information may be valuable in identifying or eliminating suspects.

Chapter 7

ORIGIN DETERMINATIONS OF SEROLOGICAL EVIDENCE

INTRODUCTION

BLOOD from any animal will result in a positive confirmatory blood identification test. For this reason all samples that are positively identified as blood must be tested further to determine the species of blood that is present. Origin determination tests can be valuable in disproving a suspect's statement. For example, a suspect may claim that the blood identified on his knife is from a deer. This story can easily be verified or disproven through the use of origin determination tests.

COMMON METHODS OF ORIGIN DETERMINATIONS

All of the origin determination tests are based on the immunological principle that all animals have an immune system that, among other things, is capable of making a protein called an antibody. Antibodies are specific proteins made to counteract foreign proteins that enter the body and will react only with the foreign protein that caused their production. The foreign protein is called an antigen. Most animals will produce antibodies against the proteins, or antigens, of other animal species. If human blood is injected into an animal of a different species, the animals will produce antibodies specifically for the proteins in human blood.

These principles are used to produce antihuman serum in rabbits. First, rabbits are injected with human blood. This causes the immune system of the rabbit to produce antibodies that are specific for the human blood. Antibodies are formed in the white blood cells of the rabbit and are then released into their serum. The rabbit serum, or liquid portion of the rabbit's blood, is then collected. This rabbit serum now contains antibodies that are specific for human blood proteins. When this rabbit serum, now called antihuman serum, comes

in contact with human blood, the antibodies will react with the proteins of the human blood. This is called a precipitin reaction and results in the formation of large antigen-antibody complexes that will precipitate, or fall out of solution and be visible.

The three common methods for using this precipitin reaction to identify the species origin of stains are the ring precipitin test, the gel diffusion method, and electrophoresis.[164] The ring precipitin test involves layering a dilute saline extract of the bloodstain on top of the antihuman serum in a small test tube. Because of the density of the antihuman serum, the blood extract will layer on top, and the two solutions will not mix, thus forming an interface between the two solutions. The test tube is then incubated, or heated, at body temperature. After heating, the tube is examined. If the stain is human blood, a white line, the precipitate, will be formed between the two layers.

The gel diffusion method is based on the fact antigens and antibodies will diffuse, or move towards each other, on an agar gel coated plate. The stain extract and the antihuman serum are placed in separate holes in the gel, where a white precipitate line will be formed between the two materials if the stain is human blood.

Another technique, used because it is more sensitive than the previous two, is cross-over electrophoresis.[165] Electrophoresis is a technique involving the use of an electric current. This electric current is passed through a gel plate, causing the movement of materials in the gel. In this method the antihuman antibody and an extract of the stain are placed in separate holes in the gel. An electric current is applied that causes the materials to move toward each other. If the stain is human blood, a white line will be formed between the two materials.

To conduct origin determination properly, controls are necessary. One such necessary control is an unstained portion of the material the blood stain is on, to verify that the blood is causing the positive reaction, and not the surface material on which it is deposited. For this reason the investigator should submit unstained samples of all the materials on which bloodstains are found.

Human blood that has been heated will often result in a negative test result. Therefore, the investigator should never dry liquid bloodstains by heating them.[166]

Chapter 8
THE INDIVIDUALIZATION OF SEROLOGICAL EVIDENCE

INTRODUCTION

THERE are many different substances in human blood that can be grouped to individualize the blood to some extent. All of these blood substances are inherited, and for this reason they are called genetic markers. The fact that these genetic markers are inherited is important because this means that they remain constant throughout a person's life. In order to gain a general understanding of these genetic markers a basic understanding of the principles of genetics is necessary. This part will begin with a discussion of the general principles of genetics and will then discuss the methods of individualizing human blood.

PRINCIPLES OF GENETICS

Genetics is the study of how the traits of an individual are inherited. Genes are the smallest unit of genetic material and specify one individual characteristic. Genes are contained on the chromosomes. Each individual has twenty-three pairs, or forty-six chromosomes. The individual inherits one-half of his forty-six chromosomes from his father and one-half from his mother, which means that each individual has two genes for every inherited trait. Some of the genes are called dominant genes, which means that when this gene is present in an individual the trait it specifies will also be present. Other genes are referred to as recessive. Recessive genes are ones in which both genes of the pair must be identical for the trait to be expressed.

THE ABO BLOOD GROUP

The ABO blood group is an example of a genetically determined individual

trait. There are three different genes that control this blood group: A, B, and O. An individual inherits one gene from each parent, A, B, or O, and the combination of the two inherited genes will determine the blood group of the individual. The A and B genes are dominant and will be expressed if present singly or in pairs. However, the recessive O genes will only be expressed if both genes of the pair are present.

These blood group genes produce antigens on the surface of the red blood cells. These antigens are protein structures that protrude out from the surface of the cell. An individual who has group A blood will have A antigens on the surface of the red blood cells; group B blood will have B antigens; and O blood will not have any antigens on the surface of the red blood cell. A person whose blood is group AB will have both the A and the B genes and A and B antigens present on the red blood cells. It is the presence and absence of these antigens that determines the blood group of an individual.

In addition to antigens on the red blood cell, individuals will also have antibodies in the serum (liquid portion) of their blood. These antibodies are specific for the antigens the person does not have. For example, a person with group A blood will have A antigens and B antibodies, an individual with group B blood will have B antigens and A antibodies, and an individual with group O blood has no antigens and both A and B antibodies present in serum.

The presence of these antigens and antibodies in a person's blood allows for the blood to be grouped. Liquid blood is easily grouped using a direct blood grouping technique. This technique involves mixing a drop of the blood with a drop of A antiserum, a drop with B antiserum, and a drop with a specially prepared H antiserum. The A antiserum will detect the presence of the A antigen on the red blood cell, the B antiserum will detect the presence of the B antigen, and H antiserum will detect the lack of antigens on the red blood cell surface, indicating group O blood.

When the antigens on the surface of red blood cells come in contact with their specific antibody, the red blood cells will be linked together in what is called agglutination. This agglutination is a clumping of the red blood cells and can be visually observed, to determine the blood group of the sample. Table 8.1 summarizes the agglutination reactions used to group human blood.

Table 8.1

A SUMMARY OF THE AGGLUTINATION REACTIONS USED TO GROUP HUMAN BLOOD IN THE ABO BLOOD GROUP SYSTEM

BLOOD GROUP	REACTION OF RED BLOOD CELLS WITH		
	A antiserum	B antiserum	H antiserum
A	Agglutination	No Agglutination	No agglutination
B	No Agglutination	Agglutination	No agglutination
AB	Agglutination	Agglutination	No Agglutination
O	No Agglutination	No Agglutination	Agglutination

When blood dries, the red cells break apart, but the red blood cell antigens are still present in the dried stains. The most common technique used to group these dried stains is absorption-elution. This method is very sensitive and can be used to group the blood from three bloodstained threads one-half inch long. For this reason it is recommended that liquid blood to be tested be absorbed on a small piece of 100 percent cotton cloth. Threads can be pulled from this cloth to group the blood.

There are other antigen systems present on the red blood cells. These antigens are unrelated to the antigens of the ABO system and can also be used to group blood. The two most common antigen systems used in forensic serology are the MN system and the Rh system. Both of these systems can be determined from liquid blood, though the detection of these antigens is very difficult in dried stains and therefore is seldom used.

A large majority of the human population (approximately 80%) are secretors. Secretors are individuals who secrete their blood group substances in their body fluids such as semen and saliva. For this reason semen and saliva stains can be analysed, and the ABO blood group may be determined. To produce an accurate analysis of a seminal stain, the forensic serologist must have a sample of the victim's saliva. This is necessary to determine whether the victim is a secretor. If the victim is determined to be a secretor, it is necessary to know the ABO blood group of the victim, as many seminal stains are mixtures of semen and vaginal secretions. If both the victim and the suspect are secretors, both blood groups may be present in a stain.

Therefore, in order to be able to state that the ABO substances found on vaginal swabs or in seminal stains originated in the semen, the ABO blood group of the victim must be eliminated. Commercial rape kits make provisions for the collection of the victim's saliva and blood for this purpose.

DETERMINATIONS OF OTHER GENETIC MARKERS OF BLOOD

In addition to the antigens on the surface of the red blood cells, there are also enzymes that are contained inside the red blood cells. These enzymes are proteins that speed up specific chemical reactions in the body. Some of these enzymes are genetically controlled and exist in different forms. This means that individuals may possess different forms of the same enzymes. These enzyme forms can be grouped from a bloodstain to further individualize the blood. The most important enzyme systems used in forensic serology are phosphoglucomutase (PGM), acid phosphatase (EAP), adenylate kinase (AK), adenosine deaminase (ADA), esterase D (ESD), glyoxalase I (GLO), carbonic canhydrase II (CA), and peptidase A (PEPA).[167] These enzymes are detected and grouped using a technique called electrophoresis. Electrophoresis is an immunological technique that involves the use of an electric field in separating proteins. The questioned stain is placed on a suitable gel plate. The plate is then subjected to an electric field. The proteins present in the stain will

migrate, or move, in different directions and distances, as a result of the electric current. The proteins can then be detected by the use of dyes.

The serum, or liquid portion of human blood, also contains proteins that can exist in several different forms. These proteins can also be identified and grouped using the same electrophoresis technique described previously.

Some of the enzyme systems that are present in blood are also present in seminal fluid. Semen stains can thus be grouped by electrophoresis and individualized to some extent.[168]

Human saliva has not been thoroughly researched to determine whether the same enzyme systems that are present in blood are also present in saliva.[169]

THE INVESTIGATIVE VALUE OF INDIVIDUALIZING BLOOD

The importance of ABO, MN, Rh, and blood enzyme genetic markers lies in the fact that they are all inherited independently of each other. Each of these genetic markers have been found to be distributed in a relatively consistent frequency in the human population. For example, approximately 43 percent of the United States population has group O blood, 42 percent has group A blood, 12 percent has group B blood, and 3 percent has group AB blood. The population frequencies have also been determined for the other genetic markers present in blood. It is the determination of several genetic markers and the comparison of their distribution frequencies that allow the forensic serologist to individualize to some extent bloodstains. These determinations can eliminate and possibly indicate suspects from the analysis of blood and seminal stains.

For example, the analysis of a bloodstain recovered from a crime scene reveals the presence of the following blood and enzyme groups: AB, PGM2, AK2-1, EAP-CA, EsD2-1. The forensic serologist can then determine the frequency of occurrence of these particular blood and enzyme groups. The population frequency of group AB is 3 percent, PGM2 is 6 percent, AK2-1 is 9 percent, EAP-CA is 3 percent, and EsD2-1 is 19 percent.[170] Because all of these groups are inherited independently of each other, the product of their frequencies equals the probability of one person possessing these five particular groups. In this case, one out of a million individuals will have these groups present in their blood. This does not constitute positive proof of whose blood is left at the scene, but if a suspect is apprehended and serological analysis reveals that he possesses the same blood and enzyme groups, this evidence is excellent in associating the suspect with the crime.

Research in forensic serology is constantly being done to isolate even more individual characteristics of human blood, underscoring again the fact that serological evidence should always be carefully collected, preserved, and submitted for analysis.

APPENDIX
ROLL CALL TRAINING TECHNIQUES

This section contains the following Roll Call Training Lesson Plans:

I. Physical Evidence
II. The Use of Luminol for Identifying Hidden Blood
III. Recording of Serological Evidence
IV. The Collection of Blood Evidence at Crime Scenes
V. The Interpretation of Bloodstain Patterns

These lesson plans are designed to be utilized in five-minute training periods during roll call.

ROLL CALL TRAINING LESSON I

Physical Evidence

Definition of Physical Evidence

Any and all objects that can establish that a crime has been committed, or provide a link between a crime and its victim, or a crime and the suspect.

Purposes of Physical Evidence

1. Often represents the decisive factor in determining the guilt or innocence of a suspect.
2. Can be a material aid in locating the suspect.

Sources of Physical Evidence

1. The scene of the crime.
2. The victim, if any, of the crime.
3. The suspect, and his environment.

Categories of Physical Evidence

1. *Physical Evidence with Class Characteristics*—A definite identification of this type of evidence can never be made, due to the possibility of more than one source of origin. Examples include; single layer paint samples, glass, fibers, hair, and blood.
2. *Physical Evidence with Individual Identifying Characteristics*—This type of evidence can be positively identified, with a person or a source of origin. Examples include; fingerprints, handwriting, bullets, and tool marks.

Potential of Serological Evidence

1. Link the scene or the victim with the suspect.
2. Establish an element of the crime.
3. Corroborate or disprove an alibi.
4. Induce an admission or confession.
5. Exonerate the innocent.

ROLL CALL TRAINING LESSON II

The Use of Luminol for Identifying Hidden Blood

Investigative Value of Luminol

Luminol is a chemical that produces a bluish-white luminescence when it comes in contact with blood. Luminol is an excellent tool to use for discovering traces of blood. This technique is applicable in situations where the suspect has attempted to clean up bloodstains. For this reason whenever a crime scene is encountered where there are no visible signs of blood, and there should be, luminol can be employed to discover hidden blood traces.

The Preparation of Luminol

Luminol is prepared by mixing the following chemicals:
sodium perborate — 0.14 grams
3-aminophthalhydrazide — 0.02 grams
sodium carbonate — 1.0 grams
These chemicals can be premixed and stored in small bottles.

The Luminol Procedure

1. Mix the premixed dry chemicals with water at the crime scene.
2. The area is darkened and the luminol is sprayed on the area to be examined.
3. The appearance of a bluish-white luminescence is indicative of the presence of blood.
4. This luminescence can be photographed and the stains can be recovered for forensic analysis.

Precautions

1. Never mix the chemicals in a metal container, as luminol will luminesce in the presence of metals.
2. A positive result does not constitute positive proof of the presence of blood, but indicates that blood may be present.
3. The effects of luminol examination are most easily seen under subdued lighting.

ROLL CALL TRAINING LESSON III

Recording of Serological Evidence

Recording Methods

1. Field Notes
2. Sketches
3. Photography

Field Notes

For every bloodstain discovered at the crime scene notes should be made that include the following information:

1. The form of the stain, liquid or dry.
2. Color of the stain.
3. Size of the stain.
4. Location of the stain—accurate measurements are necessary.
5. Estimations of the origin of the stain. These estimations should include
   a. angle of impact
   b. direction of travel
   c. possible mechanisms of its production
6. Surface the stain is on.

Sketches

Bloodstains and their accurate location should be included on all crime scene sketches. In some instances it may be necessary to compose a separate detailed drawing of a crime scene area to show the bloodstains present.

Photography

1. The camera should be held perpendicular to the stain so as not to distort the shape of the stain.
2. Photographs should include a close-up of the stain, as well as photographs that show the relationship of the stain to other objects at the scene.
3. A ruler should be included in one photograph of each stain, so as to establish a scale of measurement. This requires photographing each stain twice, one with and one without the ruler.

ROLL CALL TRAINING LESSON IV

The Collection of Blood Evidence at Crime Scenes

Investigative Value of Blood Stains

Blood can often be used to

1. identify possible suspects
2. exonerate innocent people
3. link the suspect with the scene or the victim
4. establish the events of the crime

Collection Techniques

Liquid Blood:

1. Take the blood up in a sterile medicine dropper and place it in a sterile test tube. The test tube should contain a preservative such as EDTA or an equal amount of saline.
2. Soak up the blood with a clean 100 percent cotton swatch. Air dry the swatch before packaging.

Dried Bloodstains:

1. Recover the entire bloodstained object, if possible.
2. Scrape up the dried stain with the clean razor blade. The dried stain should be scraped onto a clean piece of paper. Both the paper and the razor blade should be packaged and submitted to the laboratory for analysis.

Bloodstained Clothes:

1. Any wet clothes should be throughly air dried before packaging.
2. Each item of clothing should be packaged separately, so as to prevent transfer of bloodstains.
3. Each item of clothes should be placed between two clean pieces of paper and gently rolled up and placed in an evidence bag.
4. Care should be taken so as not to fold through any bloodstains or cuts in the material.

Important Points

1. Never package bloodstains in plastic bags.

2. Never dry bloodstains with heat or sunlight.
3. Protect all items of evidence from cross-contamination.
4. Never pack liquid blood in dry ice.
5. Always use clean packaging materials.

ROLL CALL TRAINING LESSON V

The Interpretation of Bloodstain Patterns

Physical Characteristics of Blood

1. Drops of liquid blood that fall from a wound are held together in a spherical shape due to cohesive forces.
2. These cohesive forces form a 'skin' that prevents the drop from separating while falling through the air.

Determinations of the Height of Fall of Blood Drops

1. Due to the cohesive forces drops of blood will resist splattering when striking a smooth hard surface.
2. Blood drops will splatter when they strike a porous or rough surface.
3. The only accurate method of determining the distance a drop of blood fell is to study the surface the blood struck as well as the pattern the blood produces.

Determinations of Direction of Travel

1. When a drop of blood strikes an object at an angle other than 90°, a teardrop shaped stain will be produced.
2. The sharp point of this teardrop pattern will face the direction the blood was traveling before striking the object.
3. When a drop of blood strikes an object at a very sharp angle a small droplet of blood will separate from the larger drop. This droplet will travel along the surface of the object, streaking it in a straight line. When this smaller droplet stops a rounded end will be formed. In this type of bloodstain the pointed end represents the origin, and the small rounded end points in the direction of travel.

Precautions

The preceding guidelines can only be used in making rough estimations. Precise determinations of the height of fall, angle of impact, and direction of travel should be made by an expert in a forensic laboratory.

GLOSSARY

Absorption-elution. A method used by the forensic serologist to determine the ABO blood group of a dried bloodstain.

Acid phosphatase. An enzyme found in seminal fluid that is commonly used to identify seminal stains.

Adenosine deaminase. An enzyme found in the red blood cells that can be used to individualize the blood.

Adenylate kinase. An enzyme found in the red blood cell that can be used to individualize the blood.

Amylase. An enzyme present in saliva that can be used to identify saliva stains.

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

Antigen. A substance, usually a protein, that stimulates the body to produce antibodies against it. The antigens of the ABO blood group system are found on the surface of the red blood cell and determine the blood group.

Antihuman serum. The serum of an animal, usually a rabbit, that contains antibodies specific for human blood. This serum is used to determine the species origin of a bloodstain.

Aspermia. A condition in which the male has no spermatozoa cells present in his seminal fluid.

Ballistics. The area of physics that deals with the behavior of projectiles in motion.

Benzidine. A chemical that can be used at the crime scene to identify the possible presence of blood.

Blood. A red fluid in the body that contains a set of chemical characteristics that are genetically controlled and can be used to identify a stain.

Carbonic andryonase. An enzyme, found in the red blood cell, which can be used to individualize blood.

Chromosome. A structure that contains the genes.

Class characteristics. Physical evidence that contains only enough charac-

teristics to group it into a class. A definite identification of this type of evidence can never be made, due to the possibility of more than one source of origin. Examples include hair, fibers, and blood.

Confirmatory blood identification test. An analytical procedure used in the crime laboratory to positively identify blood.

Coordinate method of measuring. A method that locates the position of an object by measuring the distance perpendicular from two reference points.

Corpus delecti. Facts of the crime.

Crime scene. The physical location where a crime occurred.

Crime scene search. A comprehensive and nondestructive accumulation, within a reasonable period of time, of all available physical evidence.

Crime scene sketch. A diagram that shows the relationship between items of evidence at a crime scene.

Criminalist. An individual who works in a criminalistics laboratory and is an expert in one area of criminalistics.

Criminalistics. The profession and scientific discipline that is directed towards the recognition, evaluation, identification, and individualization of physical evidence in law-science matters.

Cross projection sketch. A type of crime scene sketch in which the walls of the room appear folded out flat in the same plane as the floor.

Detailed drawing. A crime scene sketch that only shows one area in detail.

Electrophoresis. A technique used to identify the blood enzyme groups present in a bloodstain by separating them on a gel plate that has an electric current passing through it.

Enzyme. A protein found in the body that speeds up a chemical reaction in the body.

Erythrocyte. A red blood cell.

Erythrocyte acid phosphatase. An enzyme found in the red blood cell that can be used to individualize the blood.

Esterase. An enzyme found in the red blood cell that can be used to individualize the blood.

Fibrin. A protein found in the blood plasma, that forms a blood clot.

Flavins. A chemical present in semen that is responsible for the yellowish color of semen and causes semen to fluoresce, or glow in the presence of ultraviolet light.

Forensic science. The study and practice of the application of science to the purposes of law.

Forensic serology. The subfield of forensic science that involves all examinations of blood and body fluids (often as dried stains) associated with civil or criminal matters.

Gene. The smallest unit of genetic material to specifiy one individual trait.

Genetics. The study of how the traits of an individual are inherited.

Genetic marker. Substances in human blood that can be grouped to individualize the blood to some extent.

Glyoxalase. An enzyme found in the red blood cell that can be used to individualize the blood.

Hemastix. A commercial product that can be used in the field to make a preliminary identification of blood.

Hemoglobin. A chemical found in the red blood cell that is responsible for the red color of blood.

Leuco-malachite green. A chemical that can be used to make a preliminary identification of blood at the crime scene.

Leukocyte. A white blood cell.

Luminescence. A self-generated glow.

Luminol. A chemical that can be sprayed to search for the presence of hidden blood at the crime scene. Luminol will cause any traces of blood to luminesce in a dark room.

Oligospermia. A condition in males that involves an abnormally low sperm count.

Peptidase A. An enzyme found in the red blood cell that can be used to individualize the blood.

Phosphoglucomutasae. An enzyme found in the red blood cell that can be used to individualize the blood.

Peroxidase. An enzyme that speeds up a chemical reaction.

Physical evidence. Any and all objects that can establish that a crime has been committed, or provide a link between a crime and its victim, or a crime and the suspect.

Plasma. The liquid portion of the blood.

Platelets. A cellular component of liquid blood that initiates blood clotting.

Precipitin reaction. The formation of large antigen-antibody complexes that fall out of solution and are visible. This reaction is used to determine the species origin of blood.

Presumptive blood identification tests. A set of chemical tests that are used to indicate the presence of blood.

P30. An enzyme that is produced by the prostate gland and is used to identify the presence of semen.

Saline. A saltwater solution in which the concentration of salt is identical to that of the human body fluids.

Saliva. A fluid produced by the glands of the mouth.

Schematic drawing. A crime scene drawing that illustrates the events of an action.

Secretor. An individual who secretes his/her ABO blood group substance in his/her body fluids, such as semen and saliva.

Semen. A fluid produced by the male reproductive organs.

Seminal plasma. The liquid portion of semen.

Serology. The study of blood and body fluids.

Serological evidence. Blood, semen, and saliva stains located in association with a criminal offense.

Serum. Blood plasma that has had the clotting factors removed.

Spermatozoa. The male reproductive cell.

Tayakama. One of the tests used to positively identify blood.

Teichmann. One of the tests used to positively identify blood.

Triangulation method of measurement. Two or more widely separated reference points are chosen. The item is then located by measuring in straight lines from these reference points.

Vacutainer tube. A commercially available test tube that has a blood preservative in it.

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