UNDERSTANDING BLOOD ANALYSIS IN DUI AND TRAFFIC HOMICIDE INVESTIGATIONS

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Introduction: Analysis of blood evidence in a DUI, traffic assault, or traffic homicide case is a critical element of the case for the investigating law enforcement officer as well as the prosecutor. Blood samples taken from the defendant are a key piece of evidence in establishing criminal culpability. This document is designed to give the non-scientifically trained law enforcement officer, prosecutor, or attorney sufficient information to understand the basic properties of alcohol and blood, a basic understanding of Alabama law regarding legal issues concerning the admissibility of blood sample evidence, and how blood samples are analyzed.

Understanding Alcohol and Blood: The Basics

Alcohol: Alcohol[1] is one of the oldest substances known to mankind, but its effects are continually being studied, re-studied, and analyzed. Beverage alcohol is commonly referred to as "ethanol" or "ethyl alcohol" as well as "alcohol." Ethanol is one of a family of alcohols which includes methanol (methyl alcohol or "wood alcohol"), 1-Propanol (propyl alcohol), 1-utanol (butyl alcohol), 2-Propanol (isopropyl alcohol or "rubbing alcohol"), and ethanediol (ethylene glycol or "antifreeze").

[1] Origin of the word "*alcohol*": The *al*— in *alcohol* indicates this is a word of Arabic descent, as is the case with algebra and alkali; al- being the Arabic definite article corresponding to "the" in English. The origin of —cohol is less obvious, however. Its Arabic ancestor was *kuhl*, a fine powder most often made from antimony and used by women to darken their eyelids; in fact, *kuhl* has given us the word *kohl* for such a preparation. Arab chemists came to use *al-kuhl* to mean "any fine powder produced in a number of ways, including the process of heating a substance to a gaseous state and then re-cooling it."

The English word *alcohol*, derived through Medieval Latin from Arabic, is first recorded in 1543 in this sense. The introduction of the word "alcohol" into the English language came from French, and earlier from Medieval Latin, and is credited to a Latin translation of the works of Rhayzes (865-925), a noted Persian physician, alchemist, and natural scientist.

The invention of the distillation process to produce ethanol as a beverage is credited to Arab and Persian chemists in the eighth Century. However, the technique of distillation would not reach Europe until the twelfth century, and its name from the Arabic "al-kuhl" would become the basis for the later English word "alcohol." Arabic chemists also used *al-kuhl* to refer to other substances such as essences that were obtained by distillation, a sense first found for English *alcohol* in 1672. One of these distilled essences, known as "alcohol of wine," is the constituent of fermented liquors that causes intoxication. This essence took over the term alcohol for itself, and has come to refer to the liquor that contains this essence, as well as to a class of chemical compounds such as methanol.

Common Alcohol Compounds

Common Name	IUPAC	Formula
Methyl alcohol	Methanol	CH_3OH
Ethyl alcohol	Ethanol	CH_3CH_2OH
n-Propyl alcohol	1-Propanol	$CH_3CH_2CH_2OH$
Isopropyl alcohol	2-Propanol	$(CH_3)_2CHOH$
n-Butyl alcohol	1-Butanol	$CH_3\ (CH_2)_2CH_2OH$

Ethyl alcohol (ethanol) is a very small molecule that is completely soluble (miscible) in water. Ethanol is lighter than water. Ethanol has a specific gravity 0.789 while water has a specific gravity of 1.000. Additionally, ethanol has a boiling point at 78 degrees Celsius as opposed to water at 100 degrees Celsius. The fact that alcohol is both lighter than water and boils at a lower boiling point is essential in the distillation process. The main source of consumed alcohol is commercially prepared beverages: fermented alcoholic beverages and distilled alcoholic beverages. Beer and wine are typical fermented beverages. In both cases, a natural product (barley in the case of beer and grapes in the case of wine) is fermented by the addition of yeast microorganisms. The alcohol that is produced is the waste byproduct of the metabolism of the yeast's or bacteria's consumption of sugars found in the natural product. Throughout the remainder of this document, the terms ethanol and alcohol may be used interchangeably.

The Fermentation Process:

The understanding of alcohol must begin with the fermentation process. Fermentation of sugars by yeast is the oldest synthetic organic chemical produced by man. During fermentation, sugar is converted to drinking alcohol and carbon dioxide is released as gas bubbles. This chemical change was a great mystery to ancient man because the mixture appeared to be boiling without heat. It was not until the mid-19th Century when the noted French chemist and natural scientist Louis Pasteur discovered that alcoholic fermentation could occur only in the presence of small living "ferments" or, as they are known today, yeasts[2].

[2]Yeasts are eukaryotic microorganisms, classified in the kingdom Fungi, with about 1,500 species currently described; they dominate fungal diversity in the oceans. Most reproduce asexually by budding, although a few do so by binary fission. Yeasts are unicellular, although some species with yeast forms may become multicellular through the formation of a string of connected budding cells known as pseudohyphae, or false hyphae as seen in most molds. Yeast size can vary greatly depending on the species, typically measuring 3-4 µm in diameter, although some yeasts can reach over 40 µm.

The yeast species *Saccharomyces cerevisiae* has been used in baking and fermenting alcoholic beverages for thousands of years. It is also extremely important as a model organism in modern cell biology research, and is the most thoroughly researched eukaryotic microorganism. Researchers have used it to gather information into the biology of the eukaryotic cell and ultimately human biology. Other species of yeast, such as *Candida albicans*, are opportunistic pathogens and can cause infection in humans.

Pasteur's study on fermentation:

Louis Pasteur (1822-1895) was one of the most extraordinary scientists in history, leaving a legacy of scientific contributions which include an understanding of how microorganisms carry on the biochemical process of fermentation, the establishment of the causal relationship between microorganisms and disease, and the concept of destroying microorganisms to halt the transmission of communicable disease. These achievements led him to be called the founder of modern microbiology.

After his early education Pasteur went to Paris, studied at the Sorbonne, then began teaching chemistry while still a student. After being appointed chemistry professor at a new university in Lille, France, Pasteur began work on yeast cells and showed how they produce alcohol and carbon dioxide from sugar during the process of fermentation. Fermentation is a form of cellular respiration carried on by yeast cells; a way of getting energy for cells when there is no oxygen present. Pasteur found that fermentation could take place only when living yeast cells were present.

Pasteur was then called upon to tackle one of the most persistent problems plaguing the French beverage industry at the time, that of spoilage. Of special concern was the spoiling of wine and beer, which caused both great economic loss to the industry and tarnished France's reputation for fine vintage wines. Vintners wanted to know the cause of *l'amer*, a condition that was destroying the best burgundies.

Pasteur examined wine under the microscope and noticed that when aged properly the liquid contained few spherical yeast cells. But when the wine turned sour, there was a proliferation of bacterial cells which were producing lactic acid. It was the run-away production of lactic acid that caused the spoilage. Pasteur suggested that gradually heating the wine to a temperature range of 120 - 130 degrees Fahrenheit would kill the bacteria that produced lactic acid and allow the wine to age properly. Pasteur's book, *Etudes sur le Vin*, published in 1866 revolutionized the wine industry.

In his work with yeast, Pasteur also found that air should be kept from fermenting wine. In the presence of oxygen, yeasts and bacteria break down alcohol into acetic acid - vinegar. Pasteur conducted many experiments with yeast. Pasteur showed that fermentation can take place without oxygen (anaerobic conditions), but that the process still involved living micro-organisms such as yeast.

Pasteur's discoveries of the spoilage inherent in the natural fermentation process allowed him to develop the fundamental concept of the "germ" theory of disease transmission. While performing his experiments dealing with yeasts, and later with the silk-worm industry, Pasteur also developed what has come to be known today as sterile technique, or the boiling or heating of instruments and food to prevent the proliferation of microorganisms. Pasteur's theories of transmission of micro-organisms were gradually accepted by medical science during the decade 1870 - 1880, after work by noted British medical doctor and surgeon Joseph Lister confirmed the

germ-transmission theory of disease control in relation to infection rates in sterile and non-sterile operating settings.

In 1897, scientist Edward Buchner reported that yeasts could be broken up and that the cell-free yeast juice could ferment sugar. Later, it was found that the yeast juice contains the enzymes necessary for the conversion of sugars to alcohol and carbon dioxide. As a consequence of isolating the enzymes necessary for fermentation, mass production of beer and wine products was greatly facilitated.

The basic understanding of the potential effects of naturally occurring yeasts and other microorganisms and the subsequent collection, preservation, and testing of blood samples cannot be overstated. As will be explained later in this material, any naturally occurring yeast or micro-organism present during the collection phase of the whole blood sample may have a significant effect on the resulting reported blood alcohol concentration.

ETHANOL IN BEVERAGES

Fermented Beverages: Wine ethanol concentrations generally range from 10-12% from the fermentation of crushed grapes, but may be "fortified" by the introduction of additional alcohol during the production process. Most table wine sold in the state of Alabama is 12.5% ethanol by volume[3]. Most commonly, beer with a 3.2-5% ethanol concentration is sold at retail outlets within the state. Beer ethanol concentrations when fermented can range from 3% to as high as 15%, but are regulated by state law to not exceed 13.9% alcohol by volume[4].

Distilled Beverages: Production of distilled alcoholic beverages begins with the fermentation of one or more natural grains such as corn, wheat, rye, or barley. These grains are the source of carbohydrates (sugars) necessary for the process. The result is a wort (fermented fluid) containing up to 12% ethanol by volume, which is then distilled by heating. Alcohol (ethanol), which evaporates at 78 degrees Celsius, travels into a cooling apparatus (condenser) where it re-liquefies. The now-concentrated ethanol can be collected in a storage container, and given flavorings. Whiskey, vodka, gin, and a variety of other alcohol beverages are produced in this manner. What distinguishes the various beverage types is the carbohydrate source (grain).

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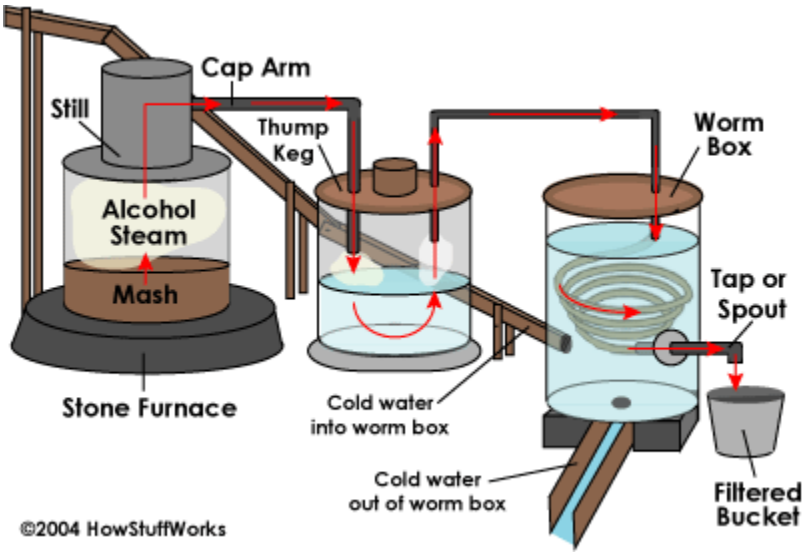
[3]The strength of alcoholic beverages is most often shown as the percentage of alcohol by volume (sometimes shown as % v/v or % ABV). This is not the same as the percentage of alcohol by weight (% w/v) since alcohol is less dense than water: 5% v/v alcohol = 3.96% by weight (w/v); 10% v/v = 7.93% w/v and 40% v/v = 31.7% w/v.

[4]See, *Code of Alabama*, 1975, section 28-3-1(3) defining "beer" as any fermented malt liquor containing one-half of one percent or more of alcohol by volume and not in excess of 13.9 percent alcohol by volume, and defining "wine" as either "fortified wine" having not more than 24 percent alcohol by volume, while "table wine" is defined as any wine containing not more than 16. 5 percent alcohol by volume. [Note: Acts of 2009, No. 09-509, amended the previous "14.9 percent" and replaced it with "16.5 percent" for the maximum alcohol content in table wine.]

In 2010, Act No. 10-607, amended the previous alcohol limitation on beer from the previous maximum amount by volume of not in excess of five percent by weight and six percent by volume to include "high alcohol" beer products for legal sale in Alabama, but retained the following requirement: "Beer or malt or brewed beverages sold by the holder of a retail beer license for off-premises consumption …. [limited to] containing one-half of one percent or more of alcohol or by volume and not in excess of five percent alcohol by weight and six percent by volume…"

Homemade distilled ethanol, commonly referred to as "moonshine", while generally having no flavoring added, possesses a fruit-like odor. The ethanol concentration in "moonshine" can range from the low 60-proof range (30% ethanol) to as high as 120-proof (60% ethanol). The name "moonshine" is derived from the nocturnal, clandestine nature of this illicit beverage production[5].

Schematic of whiskey "Still" as used in production of "moonshine" whiskey:



ETHANOL IN BLOOD

Ethanol is classified as a 'Central Nervous System' depressant (CNS) whose impairing effects are in proportion to its presence in the CNS. However, blood rather than brain tissue is the preferred representative specimen for a chemical test for impairment because blood delivers ethanol to and from the CNS and thereby is a reflection of CNS exposure to ethanol. A large body of research exists which relates ethanol concentrations in whole blood with human

[5] There are a number of statutes regulating or prohibiting the illegal manufacture of alcoholic beverages. *Code of Alabama*, 1975, section 28-1-1, makes it "…unlawful for any person, firm, or corporation to have in his or its possession any still or apparatus to be used for the manufacture of any alcoholic beverage of any kind…" Code section 28-4-2 creates the offense of possession of illegal alcoholic beverages, with the penalty being an unclassified misdemeanor. By Acts of 1915, the manufacture of illicit alcoholic beverages was made a misdemeanor, and by Acts of 1919, the manufacture of prohibited liquors became a felony. [Limited by statute to a term of one to five years imprisonment. See, Code section 28-4-24.]

The statute creating the crime of having possession of a still was adopted on September 30, 1919 with an effective date of November 30, 1919. See, Code section 28-4-50: Unlawful possession of any still or device used to manufacture any prohibited liquor or beverage. Code section 28-3A-25(9) makes it a misdemeanor offense for any person to manufacture, transport, or import any alcoholic beverage into this state except by authorization of the ABC Board.

performance. While any biological specimen may be analyzed for ethanol (blood, plasma, serum, urine, saliva, sweat, ocular fluid, tissues), results for whole blood provide an accepted, uniform standard for interpretations. For these reasons, statutes typically base *per se* limits for ethanol content in whole blood (or breath, which is a related, but different, subject and is not addressed in this publication). Forensic ethanol analyses are conducted with whole blood. ***Determining a subject's blood alcohol concentration (BAC) is the single most important issue in establishing criminal and civil liability in a judicial proceeding where alcohol is alleged to have been an element of the offense or the cause of action.***

Absorption Principles: While the entire gastro-intestinal tract (GI) is capable of alcohol absorption, almost 90% takes place in the small intestine where structural microvilli greatly increase the surface area of the gut available for absorption. With its small molecular size, ethanol readily crosses the GI tract membranes *via* passive diffusion and enters the circulation, mixing completely with the fluid portion of blood. One of the fundamental concepts in understanding blood-alcohol analysis is the fact that blood is approximately 85% water. Ethanol distributes throughout the body where it rapidly crosses back through membranes into the tissues and, most significantly, across the blood-brain barrier.

Blood: The adult human contains approximately five liters of blood, constituting about 8% of the total body weight. Whole blood is a complex, heterogeneous mixture of solid material and fluid. The solid material comprises red blood cells (erythrocytes), platelets (thrombocytes), and white blood cells (leucocytes - lymphocytes and phagocytes). Each cell type has a specific function:

• Red blood cells contain hemoglobin which binds oxygen and transports it throughout the body.

• Platelets participate in forming blood clots

• White blood cells are responsible for cell-mediated immune responses to foreign organisms

There are approximately 500 times more erythrocytes than leukocytes. The volume portion of whole blood occupied by red cells is the hematocrit (HCT), which is defined as the volume of red cells divided by the total blood volume. An average HCT for adult males is 40% to 50% and for adult females, 35% to 45%. The HCT changes with age. A low HCT indicates a relatively lower content of red blood cells in whole blood, which may be due to anemia, blood loss (internal or external) or other disease conditions.

The fluid portion of whole blood prepared by removing the cellular solids from the anti-coagulated, unclotted blood (typically by centrifugation) is called *plasma*. *Serum* is the fluid portion of whole blood remaining after the blood has clotted and the clot is removed (typically by centrifugation). Because plasma and serum contain no cellular solids, they contain a relatively greater content of water than does whole blood. ***This is significant because ethanol distributes into the various body compartments in proportion to their water content.*** In that regard, plasma and serum, with a water content of 95% to 97%, will contain more ethanol than the whole blood from which they are derived; whole blood being approximately 85% water.

This difference, 10% to 15%, highlights the importance of establishing what specimen - whole blood, serum, or plasma - was tested for ethanol before making any interpretations of the results. This issue will be discussed further in this publication.

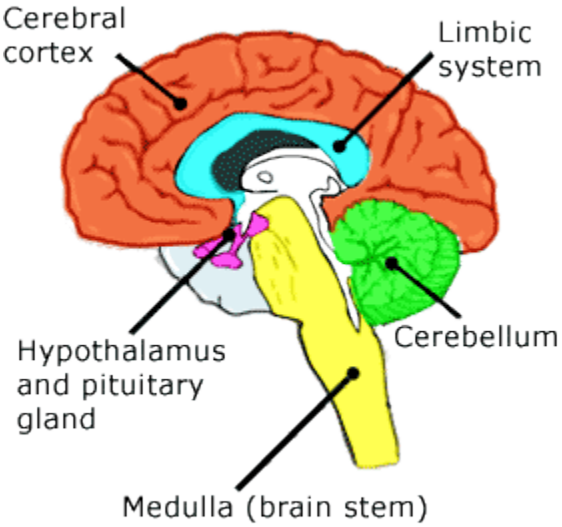
Blood alcohol concentration (BAC): Results of forensic analyses are typically expressed as a grams of ethanol per 100 mL of specimen or grams percent (g %) or simply percent (%). That a blood ethanol concentration was reported to be 0.080 g/100 mL, however, does not imply that 100 mL of blood was analyzed and 0.080 grams of ethanol were detected. The Alabama Department of Forensic Sciences (ADFS) analyzes 100 *microliters* (0.10 mL or 100 millionths of a liter) of specimen. From this volume of specimen, the actual mass of ethanol detected is on the order of 500 *nanograms* (500 billionths of a gram).

ETHANOL IN THE BRAIN

Alcohol affects various centers in the brain, both higher and lower order:

Ethanol is classified as a 'Central Nervous System' depressant (CNS), and affects the brain and nervous system quickly after it enters the blood stream. The effects of ethanol are continuous and progressive, meaning the overall effect on the CNS and on subject performance increases as the concentration of ethanol in the CNS increases. However, all centers of the brain are not equally affected by the same BAC - the higher-order centers are more sensitive than the lower-

order centers. As the BAC increases, more and more centers of the brain are depressed until all centers are depressed. The order in which alcohol affects the various brain centers is as follows:



Cerebral cortex

Limbic system

Cerebellum

Hypothalamus and pituitary gland

Medulla (brain stem)

Cerebral Cortex

The cerebral cortex is the part of the brain responsible for the highest functions of human performance. The cortex processes information from the senses, performs "thought" processing and consciousness (in combination with a structure called the basal ganglia), initiates most voluntary muscle movements and influences lower-order brain centers. In the cortex, the effects of alcohol are commonly recognized:

• Depresses the behavioral inhibitory centers - The person becomes more talkative, more self-confident and less socially inhibited.

• Impedes the processing of information from the senses - Vision can be affected at low levels of alcohol. Depth-of-field and peripheral vision are affected at BAC levels as low as 0.03% to 0.04%. General reflex response is slowed and fine motor skills are impaired at low levels of alcohol. Also, the threshold for perception of pain is raised.

• Inhibits thought process - The person does not use good judgment or think clearly. These effects become more pronounced as the blood alcohol concentration increases.

Limbic System

The limbic system consists of areas of the brain called the hippocampus and septal area. The limbic system controls emotions, learning and memory. As alcohol affects this system, the person is subject to exaggerated states of emotion (anger, aggressiveness, withdrawal) and memory loss.

Cerebellum

The cerebellum coordinates the movement of muscles. The brain impulses that begin muscle movement originate in the motor centers of the cerebral cortex and travel through the medulla and spinal cord to the muscles. As the nerve signals pass through the medulla, they are influenced by nerve impulses from the cerebellum. The cerebellum controls fine movements. For example, a sober individual can normally touch finger to their nose in one smooth motion with their eyes closed; if the cerebellum is not functioning, the motion would be shaky or jerky. As alcohol affects the cerebellum, muscle movements become uncoordinated[6]. At the approximate level of .08% to .10% blood alcohol concentration noticeable impairment can be determined through the use of properly administered field sobriety tests. In addition to coordinating voluntary muscle movements, the cerebellum also coordinates the fine muscle movements involved in maintaining balance. As alcohol affects the cerebellum, a person frequently loses his or her balance. At this stage, this person might be described as "falling down drunk."

Hypothalamus and Pituitary Gland

The hypothalamus is an area of the brain that controls and influences many automatic functions of the brain through actions on the medulla, and coordinates many chemical or endocrine functions (secretions of sex, thyroid and growth hormones) through chemical and nerve impulse actions on the pituitary gland. The hypothalamus is also referred to as the "thermostat" of the body and controls body temperature. Alcohol intoxication sufficient to depress the hypothalamus will lower the body temperature.

Medulla

The medulla (or brain stem) controls or influences involuntary functions such as breathing, heart rate, and consciousness. As alcohol depresses upper centers in the medulla, such as the reticular

[6] Field Sobriety Tests, or FSTs, are "divided attention" tests that require both physical coordination and the ability to process information simultaneously. Prior to the 1977 foundational study of field sobriety tests by Burns and Moscowitz of the Southern California Research Institute (SCRI), the Traffic Institute at Northwestern University had surveyed common sobriety tests then in use among law enforcement and prepared the "Alcohol Influence Report" form with administration of common tests of sobriety such as the "walk the line" test, "pick-up-the coins test" and the "finger to nose" test. However, research was not undertaken by the Traffic Institute to validate the relationship between alcohol impairment and ability or inability to complete the aforementioned field tests.

The SCRI field research was conducted by four large police agencies over a period of two years and involving thousands of subjects validated the use of three "standard" field sobriety tests: horizontal gaze nystagmus, the thirty second one-leg stand, and the nine step "walk and turn." The first test- horizontal gaze nystagmus- is not a divided attention test, but the observation of the involuntary movement of the eye while following a stimulus. The 1981 final report validated the three test battery to a correlation of .77 (1.00 being perfect correlation) when all three tests were used to evaluate a subject at .10% BAC or greater.

formation, a person will start to feel sleepy and may eventually become unconscious as BAC increases. If the BAC gets high enough to influence the breathing and heart rate, a person will breathe slowly or stop breathing altogether, and concurrently blood pressure will fall. These conditions can be fatal.

Stages of Alcoholic Influence/Intoxication: Kurt M. Dubowski, Ph.D., University of Oklahoma Department of Medicine, a noted authority on alcohol and the dynamics of ethanol distribution and the effects on the human body, developed a chart describing the clinical signs and symptoms resulting from the ingestion of alcohol and which is based on the blood alcohol concentration measured in grams/100 mL. Because not all centers of the brain are affected at the same blood alcohol concentrations, different subject behaviors may be visible at similar alcohol levels. This gives rise to the myth that "everyone reacts differently to alcohol." Actually, everyone reacts the same to alcohol; their CNS becomes depressed. What is different, however, is the *degree* to which each function of the CNS is depressed in each subject. The sum of these depressed functions results in the behaviors visible among subjects, which may be different. The fact that blood alcohol concentrations overlap for each clinical sign demonstrates this phenomenon.

CLINICAL SIGNS/SYMPTOMS

0.01-0.05 Subclinical
Influence/effects usually not apparent or obvious
Behavior nearly normal by ordinary observation
Impairment detectable by special tests

0.03-0.12 Euphoria
Mild euphoria, sociability, talkativeness
Increased self-confidence; decreased inhibitions
Diminished attention, judgment and control
Some sensory-motor impairment
Slowed information processing
Loss of efficiency in critical performance tests

0.09-0.25 Excitement
Emotional instability; loss of critical judgment
Impairment of perception, memory and comprehension
Decreased sensatory response; increased reaction time
Reduced visual acuity & peripheral vision; and slow glare recovery
Sensory-motor in-coordination; impaired balance; slurred speech; vomiting; drowsiness

0.18-0.30 Confusion
Disorientation, mental confusion; vertigo; dysphoria
Exaggerated emotional states (fear, rage, grief, etc)
Disturbances of vision (diplopia, etc.) and perception of color, form, motion, dimensions
Increased pain threshold
Increased muscular incoordination; staggering gait; ataxia
Apathy, lethargy

0.25-0.40 Stupor
General inertia; approaching loss of motor functions
Markedly decreased response to stimuli
Marked muscular incoordination; inability to stand or walk
Vomiting; incontinence of urine and feces
Impaired consciousness; sleep or stupor

0.35-0.50 Coma
Complete unconsciousness; coma; anesthesia
Depressed or abolished reflexes
Subnormal temperature
Impairment of circulation and respiration
Possible death

0.45+ Death
Probable death from respiratory arrest

BASIC PRINCIPLES OF BLOOD ALCOHOL ANALYSIS

Gas Chromatography

The oldest and most fundamental chemical test for intoxication is a test for ethanol in blood. Blood-alcohol analysis is commonly performed in driving under the influence (DUI) arrests and investigations of serious injury or fatal traffic accidents. This analysis is undertaken with whole blood samples collected from the suspect, as well as from any deceased driver or passenger. There are a variety of laboratory methods to determine the alcohol concentration in a biological specimen. However, the criminal justice practitioner should be familiar with the two most common methods: **gas chromatography** and **enzymatic assay**.

In General: Forensic ethanol analyses typically employ a scientific process known as *gas chromatography*, which is a widely-used technique in modern analytical chemistry. Known as a *separation science*, gas chromatography is an instrument-based technology that separates mixtures of molecules based upon their chemical and/or physical properties. The instrument is called a *gas chromatograph* (commonly abbreviated as GC). Components of all GCs include an injection device for introducing the sample mixture to the stationary medium, a stationary medium contained within the column enclosed in a temperature-controlled oven where the actual separation takes place, a carrier gas to move molecules through the stationary medium, and a device to detect the separated molecules. These components are connected in series to create a closed, tubular pathway for the molecules and gas to travel through the system.

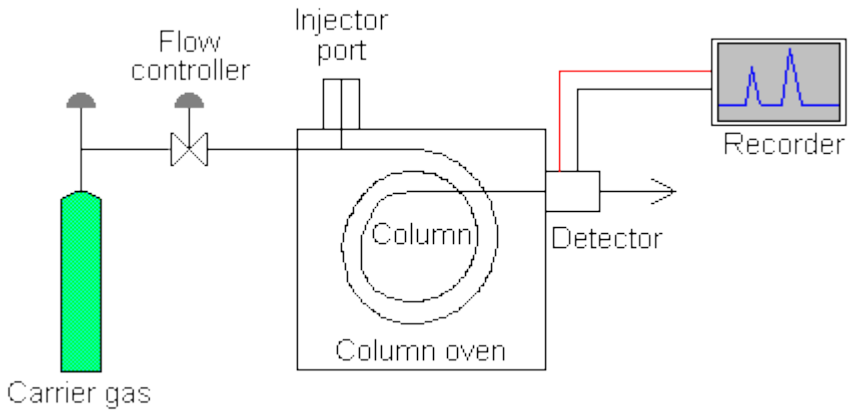
GC Operation: Separations occur with molecules in the gas state, which requires that most substances be vaporized during the analysis. This is accomplished by "injecting" *via* syringe a mixture of the molecules into a very hot (140°C to 250°C), glass-lined chamber where the molecules are vaporized into the necessary gas state. Pressurized carrier gas (typically helium) flows through this chamber and carries the vaporized molecules to a stationary, porous, inert powder such as silica packed into a long narrow stainless steel tube or glass column. This packed material is called the *stationary phase* because it remains stationary within the column as the molecules are carried through by the pressurized inert gas stream. The vaporized molecules "stick" to the stationary phase based upon their chemical or physical attraction in a process called *adsorption*. The column is then heated (hence the oven) to a temperature where molecules begin to "boil away" or dissociate from the stationary phase to be carried downstream by the gas (called the *moving phase*) in a process called *elution*.

As these molecules flow downstream, they actually undergo repeated cycles of re-adsorption and dissociation with the stationary phase in a process called *partitioning*. The stronger the attraction is between molecules and the stationary phase, the more frequently they re-adsorb and remain "stuck" and the slower they will elute from the column. This is not unlike people stepping on and off a moving sidewalk, where the speed of travel depends upon the time spent on the moving versus stationary platforms. A current alternative stationary phase to the solid porous powder packing is a waxy or resinous coating applied to the inner surface of a long coil of flexible glass capillary tubing. This coating serves the same purpose as the solid porous powder packing by

offering a surface to which molecules may repeatedly adsorb and dissociate as they flow through the column. In a properly designed system, the end result of partitioning is the elution of a succession of molecules separated into groups with similar or identical chemical and/or physical properties and, hence, structure.

Eluted molecules leave the column and flow into a detection device. The device typically used for ethanol analyses is the *flame-ionization detector* (FID), which generates an electrical signal in proportion to the mass of molecules passing through that are combusted and the ionized form detected. The successive waves of separated molecules eluting from the column and passing through the detector provide a time chart (called a *chromatogram*) which appears as a series of Gaussian (bell-shaped) peaks, each representing a group of eluted molecules. The time taken by each group of molecules to elute is called the *retention time* and is an identifiable characteristic of the molecules.

Individual substances may be quantified by measuring the size of the peaks eluting with the retention time characteristic for the substance. The GC is calibrated with a series of samples or calibrators containing known amounts of substances and establishing the mathematical detector responses for each substance separated in the mixture. This response is then used with linear regression mathematics to calculate the mass of each eluted substance. This is called the *calibration curve*.



Headspace Gas Chromatography

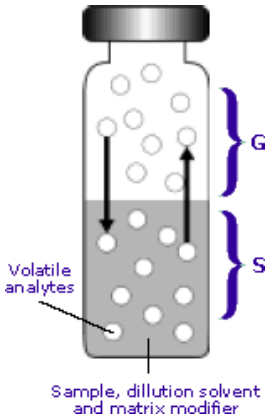
Ethanol is a small molecule that readily evaporates into a gas state at ambient temperature, even from solution in water. This volatility lends ethanol to a special type of analysis called *headspace* GC, which is a process well suited for the analysis of gases. Headspace analysis refers to the analysis of the air (head) space above a liquid or solid in a container. This is an indirect analysis because the vapors emitted from the sample are tested rather than the sample itself. Headspace GC differs from conventional GC in that a vapor mixture rather than a liquid sample is introduced into the GC. Similarly, volatile substances such as methanol, acetone and 2-propanol (isopropanol) may also be separated and measured with headspace GC.

The contents of a liquid's headspace reflects the contents of the liquid itself. A fundamental principle of science is Henry's Law (1803) which states: *"At a constant temperature, the amount of a given gas dissolved in a given type and volume of liquid is directly proportional to the partial pressure of that gas in equilibrium with that liquid."* In other words, in a sealed vessel and at equilibrium, volatile substances will be present in the vapor state above a liquid at concentrations *in proportion* to their respective concentrations in the liquid. Therefore, if a specimen is placed in a sealed vial, one may then determine the concentration of a volatile substance in the liquid by analyzing the equilibrated headspace above the liquid. With headspace GC, only volatile substances are analyzed so the potential universe of interferences is drastically limited. Further, because the non-volatile substances remaining in the specimen are not injected into the GC, the longevity of the GC column is extended and necessary maintenance reduced. Because of this factor, however, some crime laboratories will dedicate a GC solely to analysis of ethanol and attempt to operate the system without conducting periodic checks on the maintenance or repair of the GC.

Specimens are prepared for headspace GC analysis by dispensing a small volume of whole blood (typically 100 µL) into a glass vial (typically 20 mL) and adding a diluent (typically 1.0 mL of a saturated sodium fluoride solution containing 1-propanol or tert-butanol as internal standard). Vials are sealed by crimp-cap and placed into a carousel that holds many vials for a single run. The carousel is part of an autosampler that attaches to the GC and automatically samples multiple vials in sequence. (***Please refer to Appendix A***.)

The sealed vials are gently heated and mixed at 40°C to 70°C for 5 minutes to 20 minutes. During this period, volatile substances in the liquid equilibrate with the headspace in the vial above the liquid. The vial is then pressurized with carrier gas, after which the gas flow is reversed so that the pressurized vapor in the vial may flow to the column through a transfer line. The process of sample equilibration, mixing and transfer, and GC analysis is automated so the analysis may proceed unattended. Specimens are typically analyzed in batches along with quality control samples to allow monitoring of the accuracy and precision of the process.

Example of sealed vial used in GC testing:



The diluent used in sample preparation is vital to the accuracy and reliability of headspace GC. First, the internal standard, a substance of similar chemical and physical properties as the target analyte, provides a retention time marker and a scale against which the quantity of the substance is normalized. This is not unlike a ruler in a photograph providing a reference for object size. Second, the saturated salt, typically sodium fluoride, is added to promote volatilization of substances from the liquid specimen, to increase the sensitivity of the analysis. This can introduce variability to the reported ethanol concentration if the calibrators used to prepare the calibration curve are not made in whole blood prepared in an identical manner to that used in collecting the forensic specimens.

A recent innovation in blood alcohol analysis involves splitting the injected specimen vapor into two parallel capillary columns with somewhat different stationary phases and in which target substances are expected to elute with somewhat different retention times[7]. Assurance that ethanol is correctly identified and quantified is improved because the eluted peak identified as ethanol must meet the characteristic retention times for both columns in the same analysis. Whereas use of dual-capillary column headspace GC improves confidence in results, it does not render obsolete single-column analyses[8].

Reading the Chromatogram: The *chromatogram* is a graph that shows when each molecule makes contact with the detector at the end of the column. Since different kinds of molecules will reach the detector to be burned (ionized) at different rates depending on their size, shape, and other properties, the graph produced will have a series of peaks that must be read by the lab analyst. The *retention time* is the length of time it takes the separated compound to go from the injection to the detector through the gas chromatograph. The chromatogram will ideally show sharp, symmetrical peaks at different points in time representing the different kinds of molecules emerging from the column. (***Please refer to Appendix B***.)

The time it takes for a peak to appear in the known samples of ethanol is then compared with the chromatogram for the unknown sample. If a peak appears in the unknown sample at the same time that the peak appears in the known ethanol sample, then the unknown blood sample likewise contains ethanol.

***It is important to note that the area under the peak represents the concentration*.** How the lab analyst determines the area is crucial to the end result as more area represents a higher ethanol concentration. The baseline is critical in the calculation of the area as it is the boundary of that

[7] Dual column chromatography utilizes a 'Y' splitter to take the single sample from the sealed vial and to "split" the sample into two GC columns, thus allowing the scientifically approved two test analysis of the single unknown sample. For more information on GC columns, see the various GC products produced by Restek chromatography at: www.restek.com

[8] http://las.perkinelmer.com/Catalog/ProductInfoPage.htm?ProductID=BAANALYSIS

http://las.perkinelmer.com/Content/RelatedMaterials/CaseStudies/CST_GasChromaIncrAccuracyBloodAlchlAnaly.pdf

measurement. (***Please refer to Appendix D***.) As stated previously in this publication, determining a subject's blood alcohol concentration (BAC) is the single most important issue in establishing criminal and civil liability in a judicial proceeding where alcohol is alleged to have been an element of the offense or the cause of action.

The area of the peak is then compared to at least three known ethanol standards which are plotted on a graph in what is called a *calibration curve*[9]. If the known ethanol standard is a sample with . 08% ethanol and the second known standard is .16% ethanol, and the third standard is .32% ethanol, then the area of the peak from the suspect's blood sample is measured against the calibration curve from the three known standards to determine the suspect's blood alcohol concentration.

Possible Problems with Gas Chromatographs:

Problem	Cause	Solution
Peak has a flat top	Chromatogram is off scale	Sample must be diluted and retested
Peak slants	System was overloaded	Sample must be diluted and retested
Peak has a shoulder	Dirty column, or co-eluting compound	Change column and/or retest sample
Two peaks are together	Another compound has similar retention time	Change oven temperature or gas flow
Peak is very broad	Dirty inlet or column	Change inlet and /or column
Ghost peaks	Dirty column	Change column
Carry-over	Dirty inlet or column	Change inlet and/or column

(***Please refer to Appendix F showing examples of chart irregularities***.)

Hospital Analysis: Clinical and hospital laboratories also conduct ethanol determinations ***but typically do so with serum rather than whole blood***. This is because clinical laboratories are engaged in diagnostic testing, which is focused primarily on a vast universe of substances in serum. Ethanol is simply an additional analyte for testing by use of existing instrumentation. As contrasted to forensic lab testing where the GC is used, hospital and clinical laboratories use the ***enzymatic method*** to distinguish and quantify ethanol in serum. The enzymatic method is the most common chemical process in hospital laboratories. ***The main purpose of utilizing the enzymatic method rather than GC is to obtain the quickest result possible.*** A GC run may require up to eight hours while the enzymatic method can be accomplished in as short a time as 20 minutes. However, the enzymatic methods lack the exactness (accuracy) of the GC method, with an average of 10% – 20% deviation common in analysis, as well as a lack of specificity for isopropyl and butyl alcohols.

[9] See, Erwin, *Defense of Drunk Driving Cases: Civil/Criminal* at section 17.08 (Matthew Bender, 3rd Ed. 2007)

Using the enzymatic method, alcohol dehyrogenase (ADH) is an enzyme which is used to measure the concentration of alcohol in biological specimens. In the reaction, alcohol is oxidized to acetaldehyde by ADH in the presence of a coenzyme, nicotinamide adenine dinucleotide (NAD), which is reduced to NADH. [Ethanol + NAD = Acetaldehyde + NADH + H][10]

Another difference is that clinical laboratories typically express ethanol results as milligrams of ethanol per deciliter of specimen (mg/dL). This difference, however, reflects only a difference in the units of expression and not the actual content of the specimen.

Most importantly, while it is not forensically recommended to use hospital or clinical results for evidentiary purposes, if such results are employed, the impact of the different methodologies and specimen on the interpretation of the result must be examined. Blood specimens drawn in a hospital setting may produce false negative results with enzyme assays. As example, Ringer's lactate solution is typically used for fluid replacement and blood volume expander. Ringer's lactate contains lactic acid which will react with ADH analogous to ethanol thereby producing a false positive ethanol finding. Diabetics typically have acetone and isopropyl alcohol in their blood and the enzymatic test will determine both ethanol and isopropyl alcohol given an apparent BAC greater than the true value of ethanol in the blood specimen.

[10] Enzymatic testing is actually the measurement of NADH, one of the enzymes used in oxidizing the alcohol to acetaldehyde, and not a measurement of ethyl alcohol itself. Gas chromatography, by contrast, is a whole blood measuring test. Gas chromatography is preferred for the analysis of ethanol because, among many other advantages, it employs separation technology to discriminate the target analyte.

ALABAMA LAW ON CHAIN OF CUSTODY OF BLOOD SAMPLES

Who Can Draw Blood?

Under the *Code of Alabama*, 1975, section 32-5A-194 (a)(2), "only a physician or a registered nurse (or other qualified person)" is authorized to take a blood sample for use as evidence in civil and criminal cases. See, *McGough v. Slaughter*, 395 So. 2d 972 (Ala. 1981). The Court of Civil Appeals held in *Lankford v. Redwing Carriers, Inc*., 344 So. 2d 515 (Ala. Civ. App. 1977) the purpose of allowing only physicians, registered nurses, or duly licensed clinical laboratory technicians to withdraw blood samples is to ensure that standardized procedures and equipment is used, thereby preserving the validity of the test. "Strict compliance with the Chemical Test for Intoxication Act is required." *Lankford*, supra.

Alabama Code section 32-5A-194 (a)(2) mandates that only certain licensed persons may draw blood samples. By statute, all licensed physicians and registered nurses are presumed competent and qualified to draw evidentiary blood samples. The term "other qualified person" is not further defined within the Code, but several prior court decisions held that a licensed tab technologist is qualified to draw blood for evidence and subsequent analysis. See, *McGough v. Slaughter*, 395 So. 2d 972, 975; *Rehling v. Carr*, 330 So. 2d 423 (Ala. 1976); and *Powell v. State*, (515 So. 2d 140 (Ala. Cr. App. 1986). However, an EMT or an EMT-paramedic is not authorized to draw blood for evidentiary purposes[11]. It is the policy of the Alabama Department of Public Health, EMS Division, that emergency medical technicians, including EMT-paramedics, are not authorized to draw blood for non-therapeutic reasons, such as obtaining evidence for law enforcement officers. According to the Alabama Department of Public Health, the only permissible reason for an EMT to draw blood is for medical intervention and only at the specific direction of a medical provider.

In *Powell v. State*, *supra*., the defendant submitted to a blood sample drawn by a licensed medical laboratory technician. The sample was obtained under clinical conditions. Defense counsel later objected to the blood draw, but the Court specifically held the lab technician "was therefore qualified to draw blood samples" in accordance with the statute. *Powell*, 515 So. 2d at 1446. In the later case of *Ingram v. State*, 720 So. 2d 1036, 1041 (Ala. Cr. App. 1998), where the

[11] In August 2007, the Alabama Department of Public Health issued an official opinion prepared by the Compliance Coordinator for the Office of Emergency Medical Services and Trauma stating: "The ADPH legal department advised that this procedure" … [drawing blood by EMT's at the scene of an accident at the request of law enforcement officials] … "could not be performed on individuals that did not require medical interventions by on scene Paramedics. In this instance, the Paramedics would be exceeding their scope of license and would be in violation of State EMS Rules."

In August 2010, the Chief of the Highway Patrol Division of the Department of Public Safety, Major Charles Andrews, issued a Memorandum to all arresting officers of the Highway Patrol Division which stated: "It has recently been brought to the attention of the Division Chief that some troopers are requesting EMS Personnel (i.e. Emergency Medical Technicians, Paramedics, etc.) to draw blood for purposes related to an individual who is suspected/charged with driving under the influence. Such practice is not acceptable and shall discontinue immediately."

blood sample was drawn by a licensed medical technologist working as a medical laboratory technician, no objection was made to the technician's credentials or qualifications[12].

It is instructive to note that all of the above cited cases, except *Ingram*, were decided prior to the comprehensive revision of the pre-existing statute to the current 32- 5A-194, commonly known as the "Chemical Test for Intoxication Act." The original statute was enacted in 1969 and was codified at Title 36, section 155. The original statute was worded more exactly than the current statute. In the prior Title 36, section 155, in paragraph (C), the statute stated the following:

> "Only a physician, registered nurse, or duly licensed clinical laboratory technologist or clinical laboratory technician acting at the request of a law enforcement officer may withdraw blood for the purpose of determining the alcoholic content therein."

The current Code section was enacted in 1988. Upon revision, concerning the appropriate persons authorized to draw blood samples, the revised statute retained the terms "physician" and "registered nurse" but replaced "licensed clinical laboratory technologist" and "clinical laboratory technician" with the words "other qualified person." The term "other qualified person" is not further statutorily defined[13]. Presumably, the Alabama Department of Forensic Sciences has the authority under the Alabama Administrative Code to determine appropriate qualifications or set standards for credentialing for persons to meet the term "other qualified person," but as of this publication, DFS has not done so. Therefore, the term "other qualified person" is left open to the sound discretion of the trial court to determine the proper training, certification, and credentials of the individual that drew the blood sample.

Custody of the Sample:

By statute and decisional law, the state must identify the person and offer into evidence the credentials of the duly authorized person who drew the blood sample from the defendant. The blood sample cannot be presumed to have been taken in the correct manner unless the blood draw is established by the person who took the sample. The law of blood test admissibility in Alabama courts is extensive and clear: blood test evidence must be established by both predicate and chain of custody. These two requirements are properly subject to thorough cross-examination by defense counsel.

The leading Alabama case in this area regarding admissibility of the results of laboratory samples is *Ex parte Holton*, 590 So.2d 918 (Ala. 1991) which examined in detail the theory of chain of

[12] In *Powell*, the person drawing the blood sample, a Margaret Jackson, testified that she was a duly licensed laboratory technician, certified by the American Medical Technologists Registry, the National Board. She further testified that she was licensed by the National Registry and certified by the Alabama Association of Medical Technicians (See, *Code of Alabama*, 1975, section 34-18-21). The Court held she was therefore "qualified to draw blood samples in accordance with *Code of Alabama*, 1975, section 32- 5A-194(a)(2)." *Powell* was a pre-1988 decision.

[13] See, Act 88-660 which transferred supervisory authority of the state's implied consent testing program from the State Board of Health to the Department of Forensic Sciences and re-wrote and revised the state's Chemical Test for Intoxication Act.

custody[14]. In order to establish a proper chain, the State must show to a reasonable probability that the object is in the same conditions, and not substantially different from, its condition at the commencement of the chain. The court requires that proof be shown on the record with regard to exact chain of custody of the sample.

The chain of custody is composed of "links." A link is anyone who handled the item. The State must identify each link from the time the item was seized. In order to show a proper chain of custody, the record must show each link and also the following with regard to each link's possession of the item: 1) the receipt of the item; 2) the ultimate disposition of the item, i.e., transfer, destruction, or retention; and 3) the safeguarding and handling of the item between receipt and disposition. If the State, or any other proponent of demonstrative evidence, fails to identify a link or fails to show for the record any one of the three criteria as to each link, the result is a "missing" link, and the item is inadmissible. If, however, the State has shown each link and has shown all three criteria as to each link, but has done so with circumstantial evidence, as opposed to the direct testimony of the "link," as to one or more criteria or as to one or more links, the result is a "weak" link. When the link is "weak," a question of credibility and weight is presented, not one of admissibility. In this area, see also, *Lee v. State*, 748 So. 2d 904 (Ala. Cr. App. 1999)[15].

In regards to blood samples, all three Alabama appellate courts have adhered to the 'link' analysis for establishing the chain of custody. In *Creel v. State*, 618 So.2d 132 (Ala. Cr. App. 1992), a vehicular homicide case where chain of custody of the blood sample was questioned, the Court found the state did not establish a chain of custody with respect to vials of blood drawn from the defendant following an automobile accident. The transmittal forms accompanying vials upon their arrival at Department Forensic Sciences in Auburn were not signed or initialed by person who shipped blood from Dothan, and the forensic sciences investigator in Dothan who collected blood from investigating officers and placed the vials in a refrigerator with the transmittal forms could not unequivocally testify that he was person who shipped the blood vials.

The Courts generally apply a "reasonableness" test in regards to maintaining security over the blood samples. The case of *Wallace v. State*, 574 So.2d 968 (Ala. Cr. App. 1990) is instructive. In that case, the nurse on duty drew two blood samples at the hospital and handed two sealed samples to the investigating police officer. The officer then placed the vials inside a sealed Styrofoam box (referred to in the Court's opinion as 'a DUI evidence kit') in a refrigerator at City Hall where the kit remained over the weekend. The refrigerator was not locked or secured

[14] *Ex parte Holton* was later cited for authority in *Birge v. State*, 973 So. 2d 1058 (Ala. Cr. App. 2007) for the requirement that the proponent of the offered evidence must establish a strict chain of custody of samples collected for forensic analysis. See, also, *Swanstrom v. Teledyne Continental Motors, Inc*., 43 So. 3d 564 (Ala. 2009): The Alabama Supreme Court and the Court of Criminal Appeals have "consistently cited and relied on *Ex parte Holton* for its statement of the principles establishing the legal requirements for proving a proper chain of custody."

[15] *Lee* was later modified by *Pruitt v. State*, 954 So. 2d 611 (Ala. Cr. App. 2006) regarding the issue of admissibility of the state's Certificate of Analysis, but not on the issue of demonstrating the need for the chain of custody.

and was accessible to any number of city employees. The following Monday morning, the officer retrieved the still-sealed kit and delivered it to the forensics lab for analysis. The forensic analyst testified that there was nothing to indicate the kit had been tampered. The Court found the chain of custody of blood samples was sufficient despite evidence indicating some carelessness in storage of the samples[16].

The Court noted:

> "Although the evidence indicates some carelessness in the storage of the blood samples, we find that the evidence of the test results was properly admitted. '[I]t is presumed that the integrity of evidence routinely handled by governmental officials was suitably preserved "[unless the accused makes] a minimal showing of ill will, bad faith, evil motivation, or some evidence tampering." *United States v. Roberts*, 844 F. 2d 537, 549-50 (8th Cir.). Applying those principles to the facts of this case, we find that the State proved to a reasonable probability that the blood samples were the same as, and not substantially different from, the samples as they existed at the beginning of the chain. *Ex parte Williams*, 548 So. 2d 518, 520 (Ala. 1989); *Suttle v. State*, 565 So. 2d 1197 (Ala. Crim. App. 1990)."

Another example of circumstantial evidence to support the chain of custody requirement was found in *Bartlett v. State*, 600 So. 2d 336 (Ala Cr. App. 1991), the appellant's blood was drawn by a hospital nurse and the blood sample vial shortly thereafter transported to the hospital laboratory for analysis. The nurse drawing the blood labeled the vial with the appellant's name and placed the sample in a pre-vacuum sealed vial. The lab technician responsible for the analysis testified that he would not have accepted the sample for analysis had it not been in a sealed condition upon arrival at the hospital lab. The fact that a ward clerk transported the sample to the laboratory for analysis did not defeat the chain of custody. In *Bartlett*, the Court stated:

> "To establish a sufficient predicate for admission into evidence it must be shown that there was no break in the chain of custody. Identification and continuity of possession must be sufficiently established to afford ample assurance of the authenticity of the item. *Ex parte Yarber*, 375 So. 2d 1231, 1234 (Ala. 1979). 'A

[16] See a similar set of facts in *Cook v. State*, 52 Ala. App. 290 So. 2d 228 (Ala. Crim. App. 1974): The Court held that the overnight storage of blood samples in a funeral home refrigerator was not a failure in the chain of custody requirement. The funeral home employee admitted that he could not be sure that someone had not removed the samples from the refrigerator, or handled them in some way, during the night. The funeral home employee did testify that no one had tampered with the vials prior to delivery to the deputy sheriff who drove them the state laboratory the following day.

See the following related cases: *Powell v. State*, 515 So. 2d 140 (Ala. Crim. App. 1986): Sample stored in refrigerator in district attorney's office for a period of two days prior to delivery to state laboratory ; *Stone v. State*, 641 So. 2d 293 (Ala. Crim. App. 1993): Because of the late hour at which the sample was drawn, the investigating state trooper took the sealed sample home and stored the sample in his home refrigerator. In both cases, despite fact that other persons had access to the refrigerator or storage compartment, that fact alone did not cause a fatal flaw in the chain of custody.

> party need not negative the remotest possibility of substitution, alteration or tampering with the evidence.'" *Whetstone v. State*, 407 So.2d 854, 859 (Ala. Cr. App. 1981).

Likewise in *Moorman v. State*, 574 So.2d 953 (Ala.Cr.App. 1990), the Court found the chain of custody sufficient where, in prosecution for criminally negligent homicide following a fatal automobile collision, the chain of custody for a blood sample taken from the defendant was sufficiently established even though two "links" in the chain (the unit secretary at the hospital who sent the sample to the laboratory and the person from the laboratory who picked up the sample) did not testify[17]. The evidence was sufficient to establish chain of custody for victim's body, even though the person who transported the body to the morgue and the county coroner who received the body did not testify.

However, in *Suttle v. State*, 565 So.2d 1197 (Ala. Cr. App. 1990), the chain of custody was not established, and the blood sample was deemed inadmissible. The appellant's conviction for vehicular homicide was reversed because the state failed to account for the location of the blood samples drawn from the defendant during the four days between the time the samples were taken by the nurse and the time they were received by the state's forensic expert. The nurse who gave the blood samples to the trooper did not testify. The forensic analyst received the blood through the U.S. mail. The toxicologist who received the samples could not testify where the samples had been located during the previous four days. The court held it was reversible error to admit test results conducted on a blood sample when there was an insufficient chain of custody for the sample.

The importance of proving the chain of custody of a blood sample was demonstrated in *Miller v. State*, 484 So. 2d 1203 (Ala. Cr. App. 1986) where the investigating state trooper in a traffic fatality case secured blood samples from the defendant at the local hospital, then took the blood sample vials to the Jacksonville state trooper office, "put it in the envelope, sealed it and initialed it" then placed the sample in the department's outgoing mail, not the U.S. mail. Three days later, the sample was delivered to the Department of Forensic Science lab in Birmingham for analysis. There was no accounting for the location or security of the blood samples for the three days prior to delivery at the DFS lab.

Although the use of the U.S. Mail attaches a legal presumption that materials are delivered in substantially the same condition as when placed in the mailbox or post office, no such presumption is attached to "regular outgoing mail" delivery service used by a state agency. "To establish a sufficient predicate for admission into evidence it must be shown that there was no break in the chain of custody. ... Where 'missing links' are involved in the chain of custody the

[17] See, as example, the case of *Gothard v. State*, 452 So. 2d 889 (Ala. Crim. App. 1984): the Court held that conflicting testimony about when the specimens changed hands did not prevent the state from establishing a sufficient chain of custody. The chain of custody rule provides that a party seeking to introduce into evidence results of a laboratory analysis has the burden of proving that the specimen or object analyzed was, in fact, taken from the particular person alleged. Despite the conflicting testimony of the difference in time when the specimen was delivered, the state established to a reasonable certainty that there had been no substitution, alteration, or tampering with the specimen.

question presented is one of admissibility rather than credibility." Citing *Whetstone v. State*, 407 So. 2d 854, 859- 60 (Ala. Cr. App. 1981).

In the case of *Green v. Alabama Power Company*, 597 So. 2d 1325 (Ala. 1992), a wrongful death case where the defense was contributory negligence on part of the decedent, fluid samples were taken during the autopsy which, after analysis, allegedly showed the presence of a controlled substance. The plaintiff objected to admissibility of the sample where the analysis of blood and other body fluid samples were shipped by U.P.S. delivery service and subsequently analyzed at the DFS laboratory.

In *Green*, the Alabama Supreme Court held:

> "In chain-of-custody cases involving "specimens taken from the human body," the proponent of the evidence must demonstrate "where and by whom the specimen was kept and through whose hands it passed." J. Richardson, Modern Scientific Evidence, 13.14a (Ed. 1974). *Gothard v. State*, 452 So. 2d 889, 890 (Ala. Cr. App.), cert. striken, 450 So. 2d 479 (Ala. 1984)." *Suttle v. State*, 565 So. 2d 1197, 1199 (Ala. Cr. App. 1990) (reversing vehicular homicide conviction for failure of prosecution to account for blood sample during four day interval between delivery of unsealed sample to police officer and reception at laboratory.)"

The Supreme Court held in *Green* that a similar four day gap between the date of the blood draw and the subsequent delivery to the forensic laboratory, without explanation as to the sample's location or control, would render the sample inadmissible into evidence.

The case of *Jones v. City of Summerdale*, 677 So. 2d 1289 (Ala.Cr.App. 1996) is illustrative of the requirement for live witness testimony to establish both the manner of the blood draw and establishment of the chain of custody. In *Jones*, the Court of Criminal Appeals held conformity with evidentiary predicate was required for the admission of blood tests as well as compliance with chain of custody requirements.

The *Jones* case holds that results of a blood test administered to determine blood alcohol content may be received into evidence, provided a proper predicate is laid. The state must first lay a sufficient predicate in support of such evidence to indicate its reliability. A lab report indicating the results of a blood alcohol test, without any supporting testimony, invites reversible error. In *Jones*, the state did not present any testimony regarding the blood test performed on the appellant. The Court of Criminal Appeals held if the State elects to offer the results of blood alcohol test into evidence, the State must comply with the rules of evidence.

The Court's opinion stated:

> "In this case the state offered the blood test into evidence without any testimony indicating the reliability of the test, who performed the test, or the circumstances under which the test was performed. The trial court received the test without any

> foundation whatsoever having been established. The trial court erred to reversal when it incorrectly received the blood evidence into evidence. *Jones v. City of Summerdale*, 677 So. 2d at 1291.

In the case of *Nelson v. State*, 551 So. 2d 1152 (Ala. Cr. App. 1989), citing the prior case of *Kent v. Singleton*, 457 So. 2d 356 (Ala. 1984), the Court of Criminal Appeals held it fundamental to establishing admissibility that blood evidence must demonstrate the chain of custody requirement. Without establishing a strict chain of custody, the sample results are inadmissible into evidence. The evidence in the *Nelson* case did not disclose the identity of the person who withdrew the blood sample at the hospital. The trial court properly refused to admit the test results under § 32-5A-194. The results were not admissible under general evidence principles as there was no proof that the test performed on the defendant was conducted according to accepted scientific methods and there was no proof of the qualifications of the person who withdrew the blood sample. The Court further held the mere fact that the blood sample was taken at a hospital does not insure its reliability[18].

Finally, it should be noted that the Alabama Supreme Court has rejected the contention that chain-of-custody requirements in a civil action should be less demanding than the requirements in a criminal proceeding. In *Swanstrom v. Teledyne Continental Motors, Inc*, 43 So. 3d 564 (Ala. 2009), the Court summarily rejected the plaintiff's argument that a chain of custody requirement in a civil action are less strict than requirements in a criminal case. Where the toxicology report had several missing links in the custody requirement, the toxicology report was presumptively inadmissible into evidence[19].

BLOOD SAMPLE COLLECTION

In General: The proper collection of a forensic blood sample to be analyzed for use as evidence is the first critical step in establishing a proper chain of custody and most importantly to establish

[18] See in general, Annotation, *Necessity and Sufficiency of Proof that Tests of Blood Alcohol Concentration Were Conducted in Conformance with Prescribed Methods*, 96 A. L. R. 3d 745 (1979).

[19] In the *Swanstrom* case, the Court noted the toxicology report lacked any information regarding the condition of the blood samples upon receipt; whether the sample kit was sealed when received by the laboratory; who signed for and accepted the samples at the laboratory; how the samples were stored prior to testing; the date, method, and types of tests that were run on the samples; and the fact the samples were unaccounted for during the eight days between the time they were collected and the time they arrived at the lab.

the sample's integrity. Blood test evidence plays a significantly important role in determining criminal culpability in a traffic assault or traffic homicide case. In a civil action, blood evidence is likewise crucially important to determine liability where negligence is the underlying cause of action. The blood evidence taken must be properly accounted for throughout every step in the collection, storage and analysis process.

Sample Collection: Samples collected by law enforcement agencies for evidentiary purposes are usually obtained by using a forensic blood collection kit that is specifically designed to collect a forensic sample. The forensic blood collection kits (e.g. Tritech, Sirchie, Lynn Peavey) use a 10-mL gray top collection tube manufactured by BD Vacutainer®[20]. Law enforcement officers provide the kit to the phlebotomist or nurse on duty to obtain an evidentiary blood sample.

Practitioner's Note: Kits can be obtained at the manufacturer's site and they are valuable to use as exhibits and to cross-examine the person who collected the sample[21]. A typical kit should include two gray top 10-mL tubes that have the proper amounts of sodium fluoride (100 mg) and potassium oxalate (20 mg)[22], a double ended needle (20 or 21 gauge), needle holder, a non-alcohol disinfecting pad, a police officer's report, a chain of custody document, use instructions for the phlebotomist, use instructions for the police officer, a blood collection report, a consent form, evidence seals for each tube, two evidence seals for the plastic storage container for the tubes, two evidence seals for the cardboard box in which the sample is transported, a self closing plastic bag to place the kit in for safety during transport, biohazard labels, and an absorbent pad to be used when the needle is withdrawn from the draw site.

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[20]To obtain specific information concerning the vacutainer tube, access their web site: httr://www.bd.com/vacutainer click on the product FAQ's link on the left side, then scroll down to the section on common tube questions.

[21]As example, the Lyn Peavey Blood-Alcohol collection kit, #05786, can be purchased for $6.95 and consists of the following components:

Two gray top blood tubes containing 20 mg. potassium oxalate 100 mg. sodium fluoride
Needle and holder
Consent forms
Blood-collection report
Four blood-type labels for chain of custody
Providone-iodine prep pad
Four color-coded security seals
Absorbent materials
4-mil plastic Zip-Top Bag
Mailing carton
Instructions

[22] The amount of sodium fluoride and potassium oxalate in each test tube must meet the preservative and anticoagulant amounts that comply with the National Committee for Clinical Laboratory Standards standard. See publication: *Tubes and Additives for Venous Blood Specimen Collection; Approved Standard—Fifth Edition*. NCCLS document H1-A5 (ISBN 1-56238-519-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, (2003).

Blood Collection Tube Guideline:

Color Top	Additive	Required Mixing	Uses
Grey	Potassium oxalate/Sodium fluoride	8 inversions	Blood Alcohol
Yellow	SPS or SPD	8 inversions	Blood culture or DNA
Lavender	Liquid K, EDTA	8 inversions	Hematology
Red	None	None	Serum Testing

Use of the Collection Tubes: The antiseptic pad/swab/towelette used to disinfect the draw site and the package that it came in should be preserved after it is used so that it can be subsequently tested to insure that it did not contain alcohol that could contaminate the sample.

A proper evidentiary blood draw should use the draw site antiseptic that is included in the collection kit. Some medical facilities will use their own antiseptic to clean the draw site. This break in procedure could lead to sample contamination. Medical facilities that conduct routine venipunctures generally use a 70% isopropyl alcohol swab or towelette to disinfect draw sites. Other disinfectants used by hospitals can contaminate the sample as well. ***Special instructions are issued to not use isopropyl alcohol when collecting samples for blood alcohol determinations.***

Practitioner's Note: The 70% isopropyl alcohol used in skin preparation for routine venipuncture should not be used for blood alcohol determinations. Methanol can also affect results. In addition, tincture of iodine contains alcohol and likewise should not be used to clean the site. A non alcohol-containing alternative antiseptic such as chlorhexidine-gluconate or regular soap and water should be used instead. *Phlebotomy Essentials*, 3rd edition, McCall and Tankersley, page 373.

Since most medical facilities and/or phlebotomists do not draw forensic samples on a regular basis, it is unlikely they will be familiar with the proper procedures to conduct a forensic draw. It is very important to ascertain how the sample was drawn and what materials were used to prepare the site. Inspection and/or analysis of the preserved towelette (proper procedures require that the used towelette be preserved) will determine if the sample integrity has been compromised.

Some agencies and medical facilities will use a benzalkonium chloride swab/ towelette (e.g. manufactured by Triad) if it is determined that an individual may be allergic to iodine. The problem with using this type of disinfectant is that it uses alcohol as a delivery medium. The State of Colorado tested the Triad benzalkonium chloride towelette and discovered that there was alcohol in the towelette and has subsequently ordered that this product not be used to collect forensic blood samples because of the possible contamination[23].

[23]In a recent Motion to Suppress argued by the author before the Montgomery County District Court, the author determined the cleaning agent 'Hibiclens' was used by the nurse on duty to sterilize the draw site.

DFS Comment: Headspace GC analysis is capable of separating and distinguishing ethanol from isopropanol (isopropyl alcohol) and other alcohols. Accordingly, the contents of the disinfection towelette are of no consequence unless it contains *ethanol*. In such case, the applied ethanol could permeate the skin, enter the collected blood specimen and, therefore, represent a true and significant contamination of the specimen. In the absence of ethanol as the cleansing agent, no argument alleging "contamination" is scientifically justified. However, if another method was used, such as a clinical analysis employing the enzyme alcohol dehydrogenase, it is possible that the cross-reactivity of the enzyme for isopropanol occurred.

Protocol and Procedure: The person and/or agency (law enforcement agency or medical facility) that collects the forensic sample should have specific and detailed written protocols for the collection of the forensic sample.

Copies of the protocols should be obtained to determine if the collector was aware of and followed the required protocols. These protocols can be obtained from the prosecution *via* a formal disclosure request, or by issuing a subpoena *duces tecum* to the appropriate agency and/or facility that drew the sample.

Be sure to check the qualifications of the person drawing the blood. In some cases, the person drawing the blood is either not trained in blood drawing or not properly licensed. Even if licensed and meeting the statutory requirements set forth in *Code of Alabama*, 32-5A-194, the persons drawing the sample may have minimal training in this technical procedure. Physicians, nurses and other licensed medical personnel may not be aware of the proper protocols for obtaining an evidentiary sample.

Specific Protocol Required: All hospitals and other medical facilities should have specific written protocols concerning the drawing of a forensic blood sample. Most of them require that a forensic blood collection kit, provided by law enforcement, is used to collect the sample. The instructions contained in the kit are to be followed precisely and only the contents of the kit are to be used to collect the sample. Hospital supplies (antiseptic swabs/towelette, collection tubes, needles, etc.) should not be used. The forensic kits are specifically designed and equipped to facilitate a proper forensic sample collection; the use of non-authorized techniques or components will invalidate the kit's integrity and may invalidate the integrity of the sample.

DFS Comment: Blood is typically drawn pursuant to standard aseptic technique, which is to insure both the subject and the specimen are not exposed to potential pathogens. This is typical hospital policy and practice. The benefit of aseptic technique is safety to the subject and sterility

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According to the manufacturer's internet site, HIBICLENS Antiseptic/Antimicrobial Skin Cleanser is described as the following: "HIBICLENS is an antiseptic antimicrobial skin cleanser possessing bactericidal activities. HIBICLENS contains 4% w/v HIBITANE (chlorhexidine gluconate), a chemically unique hexamethylenebis biguanide with inactive ingredients: Fragrance**, *isopropyl alcohol 4%,*** purified water, Red 40, and other ingredients, in a mild, sudsing base adjusted to pH 5.0-6.5 for optimal activity and stability as well as compatibility with the normal pH of the skin." See, http://www.hibigeebies.com/sports/downloads/hibiclens_product_information.pdf

The fact that the skin cleaning agent used by the hospital to sterilize the draw site in this case contained 4% by volume isopropyl alcohol may render subsequent analysis unreliable, unless the GC run was ethanol specific.

to the specimen. There is nothing unique about an evidentiary blood draw that an otherwise competent phlebotomist must address beyond aseptic technique. Accordingly, a hospital may or may not have a specific procedure for collecting forensic specimens as this is not the hospital's mission. What is different about an *evidentiary* blood draw, however, is not the draw itself but the cleansing agent used to prior to the needle insertion; the labeling, sealing, custody and delivery of the specimen to the forensic lab; and the type of forensic examination.

Preservation and Storage: The forensic kit should contain at least two gray stopped, 10-mL, collection tubes. Gray stopped tubes will have sodium fluoride and potassium oxalate in pre-determined quantity in the tube. Sodium fluoride will preserve glucose stability for up to 3 days. If glycolysis is not prevented, the glucose concentration in a blood sample decreases at a rate of l0 mg/dL per hour. Sodium fluoride also inhibits the growth of bacteria. The tubes should also contain potassium oxalate, an anti-coagulant to prevent the blood from clotting. On occasion, the wrong tubes are used and there is no, or an insufficient amount, of the anti-coagulant and preservative in the sample. If these substances are not present, or are in insufficient quantities, or are not properly mixed, neo-genesis or endogenous production of ethanol can occur.

Practitioner's Note: Make sure the blood samples were collected in ***gray top*** tubes. Tubes with other colored stoppers may not have the proper chemicals in them. Gray stopped collection tubes contain a specific amount of sodium fluoride and potassium oxalate to prevent spoilage and coagulation of the sample. Gray stopped tubes must be mixed immediately upon collection to prevent clot formation fibrin generation. All BD Vacutainer tubes require immediate mixing following collection. The procedures used to handle the blood sample after it has been collected are another critical stage in maintaining sample integrity. If a sample is not properly mixed after collection sample integrity will be compromised. BD Vacutainer states that each gray top tube should be gently inverted eight to ten times to insure proper mixing of the anti coagulant and preservative. Failure to follow the proper mixing protocol could cause the anti-coagulant and preservative to not completely mix into the sample.

DFS Comment: Arguments over the type of collection tube involve two issues, namely the fluid consistency of the blood and artifactual production of ethanol. Gray-top tubes are one of many types of tubes used to collect specimens for clinical and chemical testing. These tubes are *preferred* when collecting specimens for ethanol determinations because (1) the anticoagulant preserves the fluid consistency of whole blood and (2) the preservative reduces the possibility of production of ethanol through post-collection fermentation. Whereas these tubes are provided in typical specimen collection kits, their use is *not mandatory*.

Collection of specimens in tubes lacking anticoagulant may give rise to a clotted specimen which, when analyzed, may resemble serum more than whole fluid blood. While it has been stated elsewhere that ethanol in whole blood rather than serum is the accepted basis for assessing performance, it has also been stated that a result in serum may be reliably "converted" to one which reflects whole blood. Furthermore, such conversion will reduce the reported result.

Accordingly, specimens collected in tubes lacking anticoagulant are acceptable for analysis and the results may be interpreted with competent expert testimony.

Collection of specimens in tubes lacking preservative presents a greater opportunity for post-collection microbiological decomposition. However, not all decomposition produces ethanol. There is equal likelihood that ethanol will remain or diminish during decomposition. Nonetheless, if decomposition does proceed with microorganisms capable of *anaerobic glycolysis* (fermentation), then ethanol may be produced which would be otherwise indistinguishable from what would otherwise be present from ingestion.

Decomposition is prevented in specimens primarily by reducing the presence of microorganisms and secondarily by proper preservation and storage which is further discussed below. While microorganisms are present in and on all humans, their numbers are limited in the blood of healthy subjects. Anything otherwise would manifest as a systemic infection (*septicemia*) requiring aggressive antibiotic treatment. Therefore, with sterile collection techniques, exposure of the specimen to potentially decomposing microorganisms is minimized. Decomposition is not uncommon in postmortem specimens because, unlike with living subjects, sterile environment for collection may not exist. Accordingly, with specimens collected with sterile techniques from living subjects, the likelihood of post-collection decomposition is minimal, if at all.

Analysis of blood samples: As stated previously, in most cases involving blood analysis, blood samples are analyzed by the gas chromatograph process. The gas chromatograph process essentially vaporizes a small portion of the sample and then that vapor is analyzed. This process can also be used to identify the presence and concentration of the anticoagulant and preservative, but most labs do not conduct these examinations unless specifically requested.

The internal standard (N-propyl alcohol) is added and mixed with the blood sample. Then, a sample of this mixture is introduced into the gas chromatograph. Usually the amount introduced is between one and ten micro-liters of solution, ideally three micro liters of solution. This is a very small amount of chemical being tested. An eyedropper yields 50 micro-liters of liquid. The laboratory will add chemicals to the sample that is being analyzed, (including N-propyl and water as a standard), that are used to draw the alcohol (or salt out) from the sample and into the vapor for analysis.

DFS Comment: Specimens are prepared for headspace GC analysis by diluting the specimen 11-fold with a solution containing saturated sodium fluoride. This equalizes the salt concentrations among all specimens regardless of what may have existed in the undiluted specimen, thereby minimizing potential variations in vaporization efficiencies.

No chemical test for anticoagulant is necessary in a forensic laboratory because its presence in a specimen is indicated by the mere fact that the specimen is unclotted. If the specimen is clotted, then anticoagulant was either absent or improperly mixed. Analysis of a clotted specimen should

be duly noted; whereupon the interpretation should consider the possibility that the specimen was serum rather than whole blood.

Storage: If the blood sample is not properly stored, neo-genesis or endogenous production of ethanol can occur in the blood sample. Simply put, the blood sample acts like a brewery and ferments producing alcohol. This process occurs because blood is a living substance. It has numerous micro organisms (yeasts, bacteria, etc.) naturally living within the blood. There is also the possibility of contamination from exterior sources during the taking phase of the blood collection process may have allowed yeasts and bacteria to enter the blood sample. During storage inside the container vial, these micro organisms consume organic materials in the blood (e.g. sugar) and produce alcohol as a waste product. This fermentation of blood may have an impact upon the later reported ethanol concentration. This "neo-genesis" type of alcohol cannot be distinguished from the alcohol suspected of being present in the driver or deceased, as compared to alcohol generated from blood decay. Refrigeration will slow down, but not prevent, the fermentation process and the production of alcohol[24].

Practitioner's Note: Gas chromatography analysis can only determine the amount of alcohol present at the time of the analysis; it cannot tell the difference between alcohol that is present in the sample due to ingestion by the sample's donor or alcohol that is in the sample due to endogenous production (fermentation) of alcohol or from some source of contamination.

Transportation of the Sample: After the sample is collected and packaged the officer will transport the sample to an evidence holding area. During the time of transport the sample will be exposed to room temperatures which are an optimum medium for bacterial growth (endogenous alcohol production). The sample should be placed into an evidence refrigerator. Refrigeration will slow these bacterial processes, but it will not stop them. The only way to stop this growth is to freeze the sample and this is generally not done because it damages the blood cells. Failure to analyze the sample within a time that is contemporaneous with the sample collection will substantially increase to possibility of sample contamination by endogenously produced alcohol. The longer the period of time between collection and analysis, the greater the possibility of contamination or endogenous production[25].

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[24] Studies have documented that temperatures are a critical factor in blood sample fermentation: *J Forensic Sci.* 1989 Jan; 34(1): 105-9. The effect of temperature on the formation of ethanol by *Candida albicans* in blood. Chang J, Kollman SE. PharmChem Laboratories, Menlo Park, CA.

The effect of temperature on microbial fermentation in blood was studied. Specimens of human blood from a blood bank were inoculated with *Candida albicans*, an organism capable of causing fermentation. A preservative was added to a portion of the inoculated specimens. These inoculated specimens, as well as uninoculated blood, were stored under various temperature conditions. Production of ethyl alcohol was monitored over a period of six months. Fermentation was found to be highly temperature dependent, with refrigeration proving to be most effective at inhibiting ethanol formation.

[25] For a good example of how <u>not</u> to handle and transport a blood sample, see the case of *Rafferty v. State*, 799 So. 2d 243, 248 (Fla. App. 2001): No refrigeration of the blood sample for eight days. The blood sample in question was taken under clinical conditions, then given to the first officer. The sample was taken to the local highway patrol station by the first officer. Rather than being secured in a refrigerated container, the sample was placed in the evidence room under unfrigerated conditions. The next day, a second officer took the blood sample from the

DFS Comment: Proper preservation and storage are necessary to insure that the condition and composition of a biological specimen at the time of analysis reflect those at the time of collection. Of particular concern is whether improper preservation and storage may lead to a change in the ethanol content.

Preservation includes aseptic collection technique and chemical supplement (sodium fluoride). While chemical preservation is preferred, the lack thereof does not necessarily result in decomposition and artifactual ethanol production. The referenced study (see footnote 24) demonstrated that negligible ethanol was produced in unpreserved blood over 3 days at 37°C and none was produced at room temperature over 10 days. Accordingly, a sterile yet unpreserved specimen is stable for these periods.

Storage considerations are primarily controlled temperature. The *preferred* storage condition for liquid blood is under refrigeration at 4°C, which is typical policy and practice in the laboratory. Refrigeration reduces biological activity, including microbiological decomposition, and evaporation of ethanol from the specimen. However, specimens stored not in the preferred manner, such as during transport to the laboratory, do not necessarily become degraded in the interim. The same referenced study (see footnote 24) demonstrated that ethanol did *not* occur at 6°C, 22°C, and 37°C unless the specimens were *inoculated* with *C. albicans*. Such intentional exposure is not encountered with specimens collected from healthy living subjects.

Degradation may also be physical (thermal). Thermal degradation typically manifests itself as a coagulated or viscous specimen which may be more difficult to dispense for analysis. This may influence the precision of the result, which is dependant upon the agreement of replicate analyses. Specimen viscosity is apparent to the analyst and is duly noted. Exposure to heat far in excess of room temperature may also promote evaporation of ethanol from the specimen (especially where a tube contains only a small volume of specimen), which may give rise to a lower result than would have otherwise been determined without such exposure. However, thermal degradation is a physical-chemical phenomenon which does *not* produce ethanol.

There is no doubt that microbiological decomposition may proceed under the right conditions, namely the right microbes, carbohydrates and temperature. Specimen decomposition manifests itself as an atypical color or consistency which may progress from a brown-green color to black sludge. There is also often a characteristic odor. These cues are apparent to the analyst and are duly noted for consideration when results are interpreted. Decomposition also generates a host of volatile, aromatic substances, some of which may appear in the chromatogram and may even interfere or co-elute with the target (ethanol) or the internal standard (1-propanol). Accordingly, microbiological decomposition sufficient to produce ethanol does not go unnoticed and undocumented in the laboratory.

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evidence room and drove the sample to the nearby crime lab that then prepared the sample for shipment *via* Airborne Express to the testing laboratory. The sample was not reported as received at the forensic laboratory until six days later. The Court's opinion stated: "This case is a textbook example of how not to handle blood samples."

OBTAINING THE CORRECT RESULT

WAS THE RESULT BASED ON WHOLE BLOOD OR SERUM?

Statutory limits in Alabama for ethanol in the blood are set at 0.02 g %, 0.04 g %, 0.05 g % and 0.08 g % in ***whole blood***[26]. Therefore, one must consider what the whole blood ethanol concentrations would have been even if another specimen was the basis for the analytical result. The most common alternate specimen for ethanol analyses is serum, which is typically analyzed in hospital and clinical laboratories. Because there is a predictable and measurable difference in water content between serum and whole blood, a result in serum may be "converted" into an equivalent whole blood result. Conversion requires a change of units from mg/dL to g/100 mL and a reduction of 10% to 15% in concentration due to the lower water content of whole blood. There are several ways to calculate this conversion. One is based upon the HCT of the subject, the theory being the HCT is a reciprocal reflection of the water content of whole blood[27].

Another method is based upon a comprehensive statistical examination of blood ethanol determined with headspace GC versus concurrent serum ethanol concentrations determined with clinical instrumentation[28]. The simplest method, however, is to reduce the serum ethanol content by the relative difference determined for parallel analyses of serum and plasma, which is generally held to be fifteen percent (15%)[29]. In this calculation, the serum ethanol result in the units, mg/dL, is divided by 1150 to provide a whole blood ethanol equivalent in g/100 mL (g%). It is also prudent to truncate the calculated result to two decimal places. This applies a bias toward a lower calculated result. For example, a serum ethanol result of 139 mg/dL would convert to 0.139 g/100 mL in *serum*, which is the equivalent of approximately 0.12 g/100 mL in *whole blood* (139 mg/dL ÷ 1150 = 0.121 g/100 mL → 0.12 g/100 mL).

Clinical reports may include interpretational notes such as "legal limit" for a serum ethanol concentration of 80 mg/dL because this result converts to 0.08 g/100 mL, a *per se* limit. However, this notation is incorrect because only the units were converted and not the ethanol concentration difference between serum and whole blood. A clinical ethanol result of 80 mg/dL

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[26] See, *Code of Alabama*, 1975, section 32-5A-191(a)(1): "A person shall not drive or be in actual physical control of any vehicle while: (1)There is 0.08 percent or more by weight of alcohol in his or her blood."

Code of Alabama, 1975, section 32-5A-191(b): "A person who is under 21 years shall not drive …if there is .02 percentage or more by weight of alcohol in his or her blood."

Code of Alabama, 1975, section 32-5A-191(c)(1): "A school bus or day care driver shall not drive or be in actual physical control …if there is greater than .02 percentage by weight of alcohol in his or her blood."

Code of Alabama, 1975, section 32-5A-191 (c)(2): "A person shall not drive or be in actual physical control of a commercial motor vehicle …if there is .04 percentage or greater by weight of alcohol in his or her blood."

[27] Shajani, et al., *Can. Soc. Forens. Sci. J.* 22(4):317-320 (1989)

[28] Barnhill, et al., *J. Anal. Toxicol.* 31(1):23-30 (2007)

[29] Charlebois, et al., *J. Anal. Toxicol*. 20(1):171-178 (1996)

is actually the equivalent of a whole blood ethanol result of approximately 0.07 g/100 mL. For a whole blood result to be 0.08 g/100 mL, a serum ethanol result could be as high as 102 mg/dL (102 mg/dL ÷ 1150 = 0.089 g/100 mL → 0.08 g/100 mL). Reliance upon the serum result without a correct and complete conversion could lead to a misinterpretation of the result.

Whole Blood or Serum? A critical question that must be asked concerning the sample that was analyzed: Was the sample whole blood or serum or plasma? This question is of particular importance if the blood alcohol determination was performed in a hospital lab.

Whole blood is composed of cellular material, plasma and fibrinogen (clotting agent). Hospitals test *serum* or *plasma* (whole blood is centrifuged or filtered to remove the cellular material and fibrinogen). Medical blood draws are primarily concerned as to whether alcohol is present and not concerned about the specific amount or concentration of the alcohol. A forensic or evidentiary blood draw is concerned with precise concentration of alcohol in the sample.

Testing plasma or serum is less cumbersome than testing whole blood, but there are problems associated with plasma (e.g. when you centrifuge the blood sample, you take the solid, cellular material out, but you leave the same amount of alcohol in a smaller volume of liquid). This process artificially increases the concentration of alcohol in the liquid-which can lead to erroneously high test result. Hospital labs test serum or plasma but report it as "blood alcohol." However, ***whole blood ethanol is not the same as serum ethanol or plasma ethanol.***

Serum is plasma with the fibrinogen (clotting material) removed. Serum is collected when the blood sample is allowed to actually clot. When the blood clots, a clear liquid (serum) forms over the blood. While serum and plasma alcohol concentrations are not significantly different, both of these tests produce results that are very different from the results produced by the analysis of whole blood. When plasma or serum analysis is used, the blood alcohol content will appear on average 14 % to 16 % higher than whole blood.

In a study of the difference between whole blood ethanol and serum ethanol and plasma ethanol reported in the *Journal of Analytical Toxicology*, Vol. 11, November/December 1987, "*Comparison of Plasma, Serum, and Whole Blood Ethanol Concentrations*" by forensic scientists Charles L. Winek and Mark Carfagna, fifty subjects who had consumed alcohol were tested by taking a whole blood sample and simultaneously taking a second sample for blood serum. The average relative difference in reported ethanol concentration between whole blood and serum or plasma was 1.14 ± 0.02 across all fifty subjects. In other words, the plasma-serum series of tests showed a higher ethanol result by 14% as compared to the whole blood tests, with both samples measured by direct injection gas chromatography.

As Winek and Carfagna noted: "A person with an ethanol concentration of 92 mg/dL in whole blood could have a reported concentration above 100 mg/dL if either serum or plasma is analyzed." In other words, according to Winek and Carfagna, a reported hospital lab result of 0.10 % BAC [mg/dL] using blood serum would be actually 0.092 % BAC if whole blood was analyzed. In view of the significant penalties for a conviction of alcohol related traffic collision,

it is imperative that competent counsel understand which method — whole blood or blood serum/blood plasma- was used to determine the subject's reported blood alcohol level.

A second scientific treatise, *Clinical Chemistry*, Volume 39, No. 11, 1993, confirmed the earlier Winek and Carfagna study by running tests of blood samples taken from 211 persons. Professor Petrie M. Rainey of the Department of Laboratory Medicine, Yale University School of Medicine, using the gas chromatography method of analysis at the Yale University School of Medicine laboratory facility, determined the median ratio to be 1.15. The common range of deviation among the 200+ individuals examined was 1.14 to 1.17.

The report noted to a 95% degree of confidence, the range was 1.14 to 1.17, and the median ratio of all subjects was 1.15. As Professor Rainey noted:

> "Clinical laboratories have traditionally measured ethanol concentrations in serum or plasma. All state laws that define driving while intoxicated are written in terms of whole-blood concentrations. Because treatment of injuries takes precedence over collection of evidence, alcohol concentrations obtained in the emergency department are often the only measurements available on injured motorists. These measurements may be used as legal evidence in both civil and criminal proceedings. However, differences between serum and whole-blood alcohol concentrations have created difficulty in interpreting serum concentrations under legal statutes."

Professor Rainey's study determined, on average, the correct method to convert serum or plasma into the grams per cent by weight calculation was to divide by 1.15. "The median whole-blood alcohol concentration can be calculated by dividing the serum alcohol by 1.15 for a result in mg/dL or by 1150 for a result in weight percent." However, this computation is not precise for all persons, and there is a degree of variance in the concentration of ethanol between whole blood and blood plasma. *Clinical Chemistry*, Vol. 39, No. 11 at pages 2288-2292.

For Additional Information:

There are two publications that are recommended reference material in understanding alcohol-related evidence in civil and criminal trials and legal proceedings. Both publications are recommended for law enforcement, defense attorneys, and prosecutors:

1) ***Garriott's Medicolegal Aspects of Alcohol, 5th Edition*** (2008)
by James C. Garriott, Ph.D, et. al.
Available from: Lawyers and Judges Publishing Company, P.O. Box 30040, Tucson, Az. 85751
(800) 209-7109

2) ***Attacking and Defending Drunk Driving Tests*** (Rev. 2 3/10)
by Donald J. Bartell, Mary Catherine McMurry, and Anne D. ImObersteg
Available from: James Publishing, Inc., 3505 Cadillac Ave., Suite H, Costa Mesa, CA. 92626
(800) 440-4780

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Mr. Mahaney received his B.A. from The Citadel, Charleston, S.C. and his law degree from Jones School of Law in Montgomery, Alabama. He was admitted to the Alabama state bar in 1989 and is member of the bar of the state of Alabama and the federal courts, to include the U.S. Supreme Court.

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Dr. Valentine holds B.S. degrees in both Biology and Chemistry from Centenary College of Louisiana and received his M.S. and Ph.D. degrees from The University of Mississippi in Medicinal Chemistry. In 2008, Dr. Valentine retired from the University Of Arkansas College Of Medicine where he served for 19-years as Professor of Pediatrics, Pharmacology, and Myeloma Research.

Prior to Dr. Valentine's tenure at the University of Arkansas, Dr. Valentine was a faculty member at the University of Missouri-Kansas City School of Medicine (1973-1978) and Oral Roberts University School of Medicine in Tulsa, Oklahoma (1978-1990) where he served as Professor and Chairman of Pharmacology from 1984-1990. He was recognized as the Outstanding Faculty Member, an award bestowed by the medical students in 1984.

At all the academic institutions where Dr. Valentine served as a faculty member he also directed certified toxicology laboratories and had active research programs in the fields of drugs of abuse, performing and developing analytical techniques for determining drugs of abuse, and correlating levels found in humans with behavioral effects. Dr. Valentine has published 60 papers in the scientific and medical literature and has written 12 chapters in professional treatises and is co-author of 3 books. Dr. Valentine has presented numerous training seminars on the correlation of drug use and behavioral effects in relationship to levels determined in physiological fluids both in living and post-mortem situations. Dr. Valentine is Board certified in applied clinical pharmacology and as a forensic examiner. He is also a Certified Flight Instructor and active pilot. Dr. Valentine has been certified as an expert witness in numerous civil and criminal trials and administrative hearings concerning drug findings. Dr. Valentine now serves as a consultant in medical pharmacology and toxicology.

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