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Examination and Analysis of John Wesley Hardin Death Scene Bullet

James A. Bailey, Kurt House, Maher Noureddine, and Erwin Vermeij

The purpose of this research was to examine a bullet collected immediately following the 1895 shooting death of John Wesley Hardin. The bullet was stored in a corked glass vial cushioned by a ball of cotton. Limited details were known about the glass vial, cork, cotton and bullet. Other unknowns included storage conditions of the bullet or the number of times the bullet was removed from the glass vial and handled by previous owners or guests of the owners.

While forensic science was in its infancy in 1895, ongoing scientific advances fostered forensic developments which could be used to analyze the Hardin death scene bullet. First, during the same year of Hardin's death, 1895, Wilhelm Conrad Roetgen discovered X-rays at the University of Wurzbürg in Germany. Subsequent to Roetgen's discovery of X-rays, Manfred von Ardenne, a German physicist, invented the scanning electron microscope (SEM).[1-2] And twenty-four years prior to von Ardenne's invention of the SEM, in 1913 Victor Balthazard, a professor of forensic medicine at the Sorbonne in Paris, made a significant contribution to firearms identification. Balthazard published an article on his research on how to identify bullets, firing pin impressions, and observations on breech face, ejector and extractor marks.[3-4] Then there have been relatively recent advances in forensic science which resulted from the 1953 discovery of the molecular structure of Deoxyribonucleic acid (DNA). And contributions from researchers James Dewey Watson, Francis Crick, Rosalind Franklin and Maurice Wilkins solved the structure of the DNA molecule which appears like a twisted ladder, known as the double helix.[5-6] Due to these forensic inventions and discoveries, involving x-rays, firearms and DNA, a twenty-first century forensic examination of nineteenth century evidence from Hardin's death scene is possible.

After Hardin's death R.B. Stevens displayed the bullet in a glass vial at the Acme Saloon, El Paso, Texas. In 1975, the bullet was in the possession of a collector, Bob McNellis.[7] Subsequently, in 1996 the glass vial and bullet were recorded in the estate of Dr. Richard C. Marohn.[8] Today, the bullet is in Bill Koch's collection at his western town in Gunnison County, Colorado. On October 14, 2017, the bullet was removed from the glass vial and examined in a building designated as the sheriff's office.

Researchers used state-of-the-art forensic science technology to learn more about the bullet in this shooting case. Four types of examinations were performed: first, a macroscopic examination to assess bullet morphology and collect measurements; second, a digital microscopic examination to evaluate striations on the bullet's lands and grooves; third, a Scanning Electron Microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDS) to examine traces of residual material on the surface of the cotton fibers; and finally, DNA analysis of swabs used to collect biological material from the bullet, inside surface of the glass vial and cotton fibers.

Hardin's death can best be summarized by examining newspaper articles written by late 19th century reporters. Reporters claimed Hardin, a Texas desperado, killed twenty-seven men while Hardin claimed he killed forty-two men during his escapades.[9] Even so, these forensic analyses provided new information to the research, articles and books written about his death.

In 1877, Hardin was convicted of murdering Deputy Sheriff Charles Webb. Hardin was tried, convicted and sentenced to twenty-five years in Huntsville Penitentiary in Texas. While in prison Hardin studied law and when he was released, Texas Governor James Stephen "Big Jim" Hogg granted Hardin a full pardon thereby allowing him to practice law. After being released from prison, he moved to El Paso, Texas where he opened a law office. In 1895 an El Paso policeman, John Selman, Sr. and Hardin got into a dispute and threats were exchanged between the two men.

Prior to becoming a policeman, Selman was also a character of dubious distinction and had killed twenty-nine men during his career. On August 19, 1895, Hardin was standing at the bar in the Acme Saloon playing dice when Selman entered the saloon and shot Hardin in the back of the head.[10-11] Hardin received other gunshot wounds to his body from Selman; however, physicians S. G. Sherard, W. N. Vilas and Alward White concluded the bullet to the head was the fatal shot. The bullet entered the rear base of Hardin's cranium and exited his left eye.[12] In the aftermath of the shooting, R. B. Stevens, the proprietor of the Acme Saloon, discovered a bullet on the floor and collected it as a souvenir. Stevens stored the bullet in a corked glass vial cushioned by a ball of cotton.[13]

Revolver and Shots

John Selman used a .45 caliber Colt Peacemaker serial # 141805 the night of the fatal shooting.[14] The revolver was confiscated after the shooting and held as evidence. Selman was charged with Hardin's death and the first trial ended with a hung jury. However, Selman met his demise before the court could schedule a re-trial. Selman's revolver remained at the El Paso courthouse until about 1907 when Hardin's nephew, Mannie Clements, purchased it for $24. Clements sold the revolver the same day for $100 to Tom Powers who displayed it at his Coney Island Saloon in El Paso, Texas.[15] The .45 caliber Colt Peacemaker is currently in James Earle's collection of western memorabilia.[16]

Selman fired four rounds all of which struck Hardin. The "1st shot passed through Hardin's cranium and exited his left eyelid; the 2nd hit his little finger on his left hand; the 3rd entered his upper right breast and the 4th struck his right arm."[17] It is unknown which one of the four bullets fired was collected by R. B. Stevens; however, on August 20, 1895, a journalist with the *El Paso Herald* reported Stevens' account of one of the bullets fired, "The bullet that passed through Hardin's head struck a mirror frame and glanced off and fell in front of the bar at the lower end."[18] Based on Stevens' eyewitness account of the shooting, the bullet he collected may have been the one that passed through Hardin's cranium.

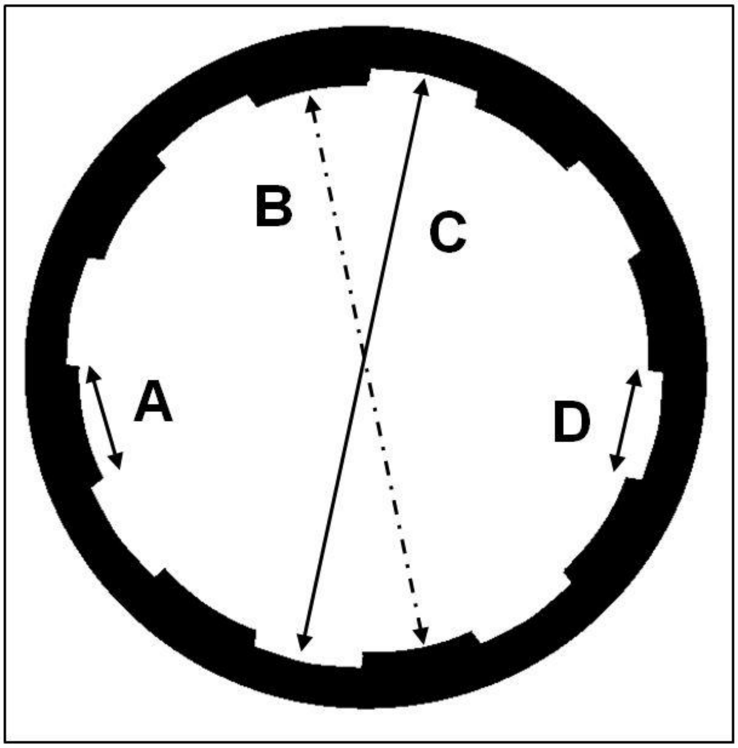
Class and Individual Characteristics of a Bullet

Fired bullets contain distinctive markings. When Selman fired his revolver, the bullet passed through the gun barrel and the surface of the bullet came into contact with the lands and grooves in the barrel. The lands and grooves in the barrel of a firearm are referred to as rifling. Manufacturers cut helical rifling into the barrel during production which results in a right or left twist on the bullet. The helical design of the rifling imparts a spin on the bullet which increases stability as the bullet travels out the barrel toward a target.[19-20]

A fired bullet has impressions identified as lands and grooves on the bullet and the impressions are mirrored on the bullet. Lands in the barrel leave grooves on the bullet and grooves in the barrel leave lands on the bullet. Seven properties: the diameter of the bullet, number of lands and grooves, direction of twist, rate of twist, width of the lands, width of grooves, and cannelures, which hold bullet lubricant and serve as a guide for adjusting the crimping die in loading ammunition, are examples of class characteristics. Class characteristics generally organize objects that are similar in shape and size.[21]

If there are six lands and six grooves in the barrel of a revolver and a bullet in question only has four lands and four grooves, the bullet in question could not have been fired from that revolver because the class characteristics are different. On the other hand, if the barrel of a revolver and a bullet in question has the same number of lands and grooves, the bullet could have been fired from the revolver because the class characteristics are the same.

Typically, the caliber of a handgun is the diameter measured from land to land in a barrel. The diameter measured from groove to groove in a barrel generally corresponds with the bullet diameter recommended for the firearm. Lead bullets may be cast slightly oversized to prevent gases from escaping as the bullet travels down the barrel.[22]



Rifled Barrel with 8 Lands and 8 Grooves: A = Land Width; B = Land to Land Diameter; C = Groove to Groove Diameter; D = Groove Width.
(Authors' Collection)

When bullets are examined microscopically, the lands and grooves typically display linear striations which are parallel to the long axis of the bullet. The linear striations are individual characteristics that allow firearms examiners to match bullets fired through the same barrel. So if two bullets are fired through the same barrel, they will have the same linear striations when aligned in a comparison microscope. Therefore, when fired from a different barrel, the striations will not match.[23] Moreover, when revolver barrels are manufactured, the rifling cutting process leaves microscopic imperfections in the barrel from the tool that cuts the rifling. These linear striations are of various thicknesses on a bullet when fired. The rifling process constantly changes due to wear on the cutting tool so each barrel is unique.[24]

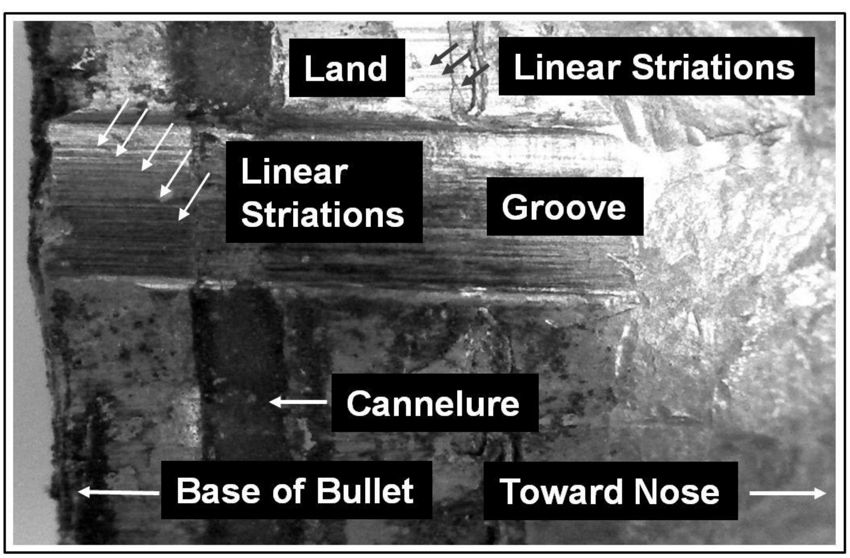
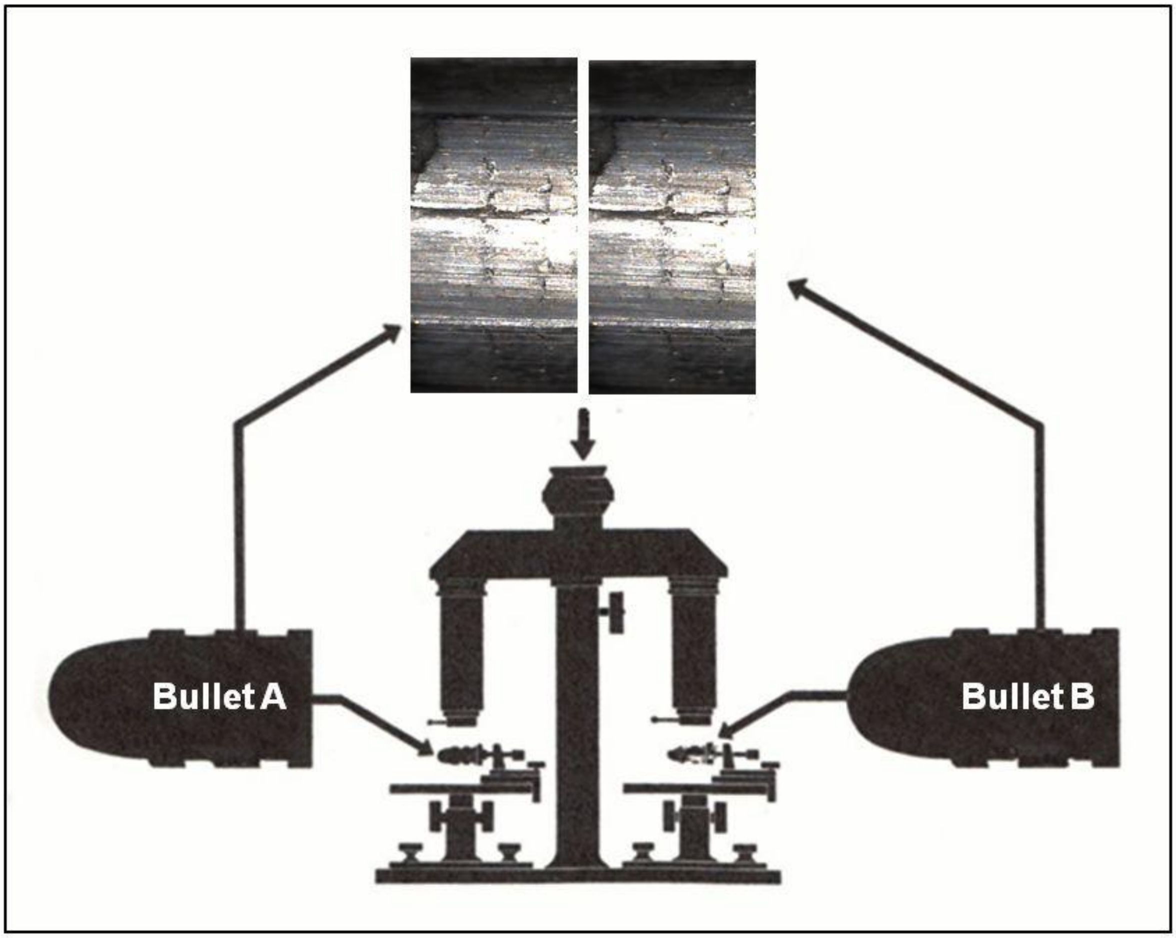


Diagram of .45 Caliber Bullet with Lands, Grooves, and Linear Striations.
(Authors' Collection)

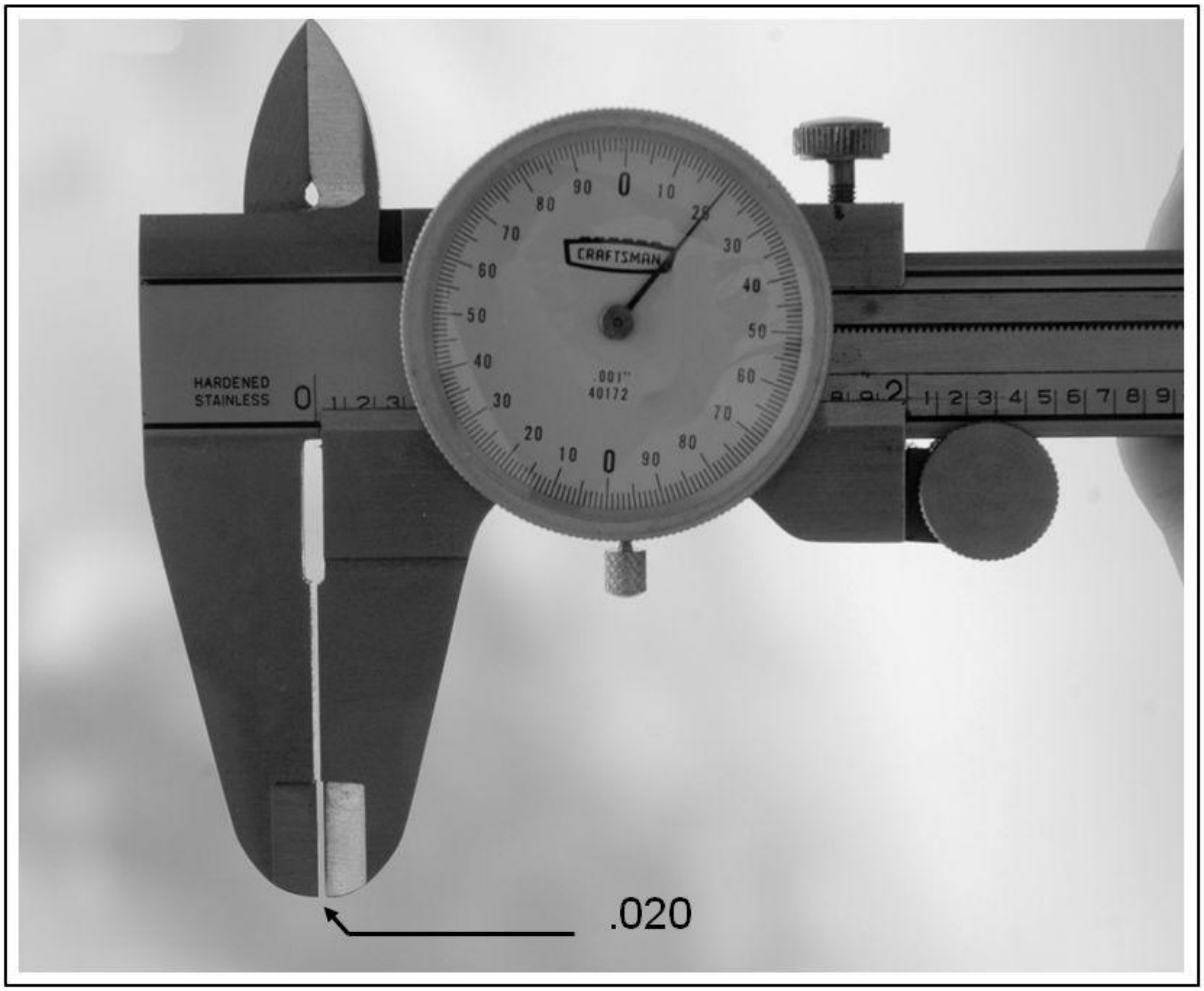
Firearms examiners have been examining the individual characteristics of linear striations as a method of matching bullets since the 1920s using a comparison microscope.[25] And today, firearms examiners continue to use a comparison microscope for matching the striations of two bullets. A comparison microscope allows an examiner to view the striations of two bullets simultaneously. When a bullet is recovered from a shooting scene and a revolver that is believed to have fired the shot is identified, a firearms examiner test fires the revolver, collects the bullet that was known to have been fired from the revolver and compares it to the bullet collected at the scene. If the striations on the lands and grooves match, it can be concluded that the revolver in question fired the bullet at the scene.[26]



Comparison Microscope.
(Authors' Collection)

On October 14, 2017, the bullet collected at the Acme Saloon in 1895 was examined for class and individual characteristics. The bullet was stored on a ball of cotton in a corked glass vial measuring 2.6 inches by 1.3 inches. Bullet style was a lead round nose flat point (RNFP) with a hollow base. Average bullet diameter measured 0.451 inches and it weighed 231.48 grains. It was noted the bullet had two cannelures, one of which was located 0.118 inches from the base of the bullet and the other was 0.299 inches from the bullet's base and average thickness of the cannelures was 0.017 inches. The nose and base of the bullet were deformed.

The Acme Saloon bullet measurements were compared to five vintage .44 and .45 caliber bullet measurements to determine the differences between the two calibers. Two calibers were selected to compare to the Acme Saloon bullet because some news articles reported that Selman used a .44 caliber revolver.[27-28] The vintage bullets were taken from five .44 caliber and five .45 caliber cartridges with different head stamps. Measurements of the cartridges were taken and the difference between the .44 and .45 caliber bullet diameter was 0.020 thousandths inches when compared to the largest .44 vintage bullet measurement and the smallest .45 caliber vintage bullet measurement. There was only a 0.002 difference between the average of the .45 caliber vintage bullets when compared to the bullet collected at the Acme Saloon. There was 0.036 thousandths inches difference in diameter between the Acme Saloon bullet and the .44 caliber vintage bullet. Even though the length of the Acme Saloon bullet measured 0.667 thousandths inches and the vintage .45 caliber bullet measured 0.710 thousandths inches, the difference in length could be attributed to its deformity. Therefore, the bullet collected from the Acme Saloon is consistent with the measurements of a .45 caliber bullet.



Dial Calipers illustrating difference between .44 and .45 caliber bullet diameters.
(Authors' Collection)

An accurate measurement of Hardin's entrance wound, the bullet mark impressions in the floor as well as the bullet mark on the mirror are unavailable. However, it is unlikely a differentiation could be made between a bullet hole made between a .44 and .45 caliber bullet primarily because of the 0.020 thousandths inch differences between the .44 and .45 caliber bullets. When bullets travel through different types of substrates, the diameter of the hole can vary greatly and bullets with similar diameters cannot be differentiated.

.44 Caliber Bullet Weights		.45 Caliber Bullet Weights	
Head Stamp		Head Stamp	
Peters	200.8	Peters	252.2
REM-UMC	199.7	UMC	248.7
WRA-CO	194.5	W.C.CO.	254.5
US-WCF	194.1	W R A Co	251.5
No Markings	198.9	No Markings	250.3
Av. Wt.	**197.6**	**Av Wt.**	**251.44**

A comparison of vintage .44 and .45 caliber bullet weights.

The weight of the Acme Saloon bullet, 231.48 grains, was also consistent with the weights of vintage .45 caliber bullets. The heaviest .44 caliber vintage bullet was 200.8 grains while the lightest .45 caliber vintage bullet was 248.7 grains. Therefore, the weight for this bullet style could be used to differentiate between a .44 and .45 caliber bullet.

Powder weights of the vintage bullets were measured; however, the weight of all the vintage cartridges could not be obtained. The bullets were pulled using an inertia bullet puller and only four out of ten cartridges yielded granular powder that could be weighed, two from the .44 and two from the .45. Powder contained in six of the cartridges was compacted into a solid mass and could not be extracted from the cartridges for an accurate weight. However, from the average weight of the two .44 caliber cartridges and the average weight of the two .45 caliber cartridges, the average powder weight was 37.5 and 34.7 grains, respectively. One source reported that the .45 Colt cartridge was originally loaded with 40 grains of FFg powder and a 255 grain lead bullet. The velocity for this load would be about 900 feet per second (fps).[29-31] Based on this velocity and bullet weight, the kinetic energy (KE) was calculated to be 459 foot pounds or 622 joules.[32]

Compacted Mass of Black Powder.
(Authors' Collection)

.44 Caliber Powder Weights		.45 Caliber Powder Weights	
Head Stamp		Head Stamp	
Peters	*	Peters	*
REM-UMC	*	UMC	*
WRA-CO	34.1	W.C.CO.	33.1
US-WCF	*	W R A Co	36.3
No Markings	40.9	No Markings	*
Av. Wt.	**37.5**	**Av Wt.**	**34.7**

A comparison of vintage .44 and .45 caliber powder charges.

The Acme Saloon bullet had morphological characteristics of a ricochet bullet. The nose and base of the bullet were deformed. The bullet was more elliptical than round and the measurements of the bullet diameter ranged from 0.390 to 0.449 thousandths inches. Several variables can affect bullet deformation and ballistic researchers have noted, "The degree of deformity varies depending on the texture of the bullets, the angle of impact and the consistency of the object which is struck."[33-34] The bullet deformation was likely caused by the bullet striking a hard object. However, a microscopic examination of the bullet did not reveal any particles of transfer to the nose or base of the bullet. Therefore, the objects the bullet came into contact with could not be identified.

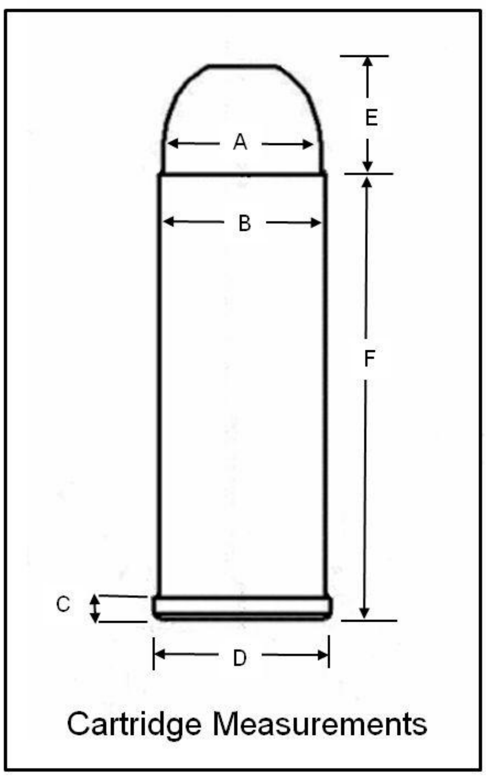
The rounds fired by Selman did have sufficient energy to penetrate or perforate Hardin's body. U.S. military tests report that a 150 grain bullet can penetrate the skin with approximately 5 fps of kinetic energy or a bullet velocity of 125 to 150 fps.[35] Dr. V. J. DiMaio, a forensic pathologist, noted that a velocity of 230 fps will penetrate skin and damage subcutaneous tissue and muscle.[36]

Base Deformation of Acme Saloon Bullet.
(Authors' Collection)

Nose Deformation on Acme Saloon Bullet.
(Authors' Collection)

.44 Caliber Vintage Bullet with Solid Base.
(Authors' Collection)

.45 Caliber Vintage Bullet with Hollow Base.
(Authors' Collection)



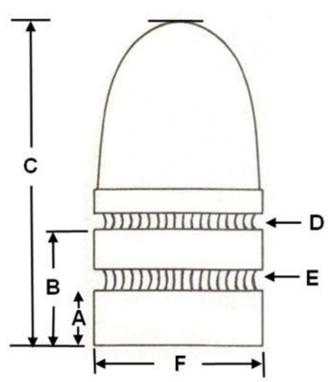
Cartridge profile: Location of measurements Recorded.
(Authors' Collection)

Vintage .44 Caliber Cartridge Measurements						
Head Stamp	A	B	C	D	E	F
Peters	.415	.438	.060	.516	.275	1.305
REM-UMC	.420	.438	.059	.513	.303	1.307
WRA-CO	.415	.449	.059	.510	.300	1.305
US-WCF	.406	.438	.056	.511	.314	1.316
No Markings	.421	.438	.055	.518	.302	1.300
Av.	**.415**	**.440**	**.058**	**.514**	**.299**	**1.307**

Table of measurements taken from vintage .44 caliber cartridges

Vintage .45 Caliber Cartridge Measurements						
Head Stamp	A	B	C	D	E	F
Peters	.455	.475	.060	.504	.326	1.269
UMC	.454	.477	.059	.505	.335	1.265
W.C.CO.	.441	.476	.055	.510	.306	1.279
W R A Co	.456	.477	.059	.510	.310	1.285
No Markings	.457	.477	.062	.512	.329	1.275
Av.	**.453**	**.476**	**.059**	**.508**	**.321**	**1.275**

Table of measurements taken from vintage .45 caliber cartridges



Bullet Measurements

.45 Acme Saloon Bullet Dimensions in Thousandths Inches		.45 Caliber Bullet Dimensions in Thousandths Inches	
A	0.118	A	0.095
B	0.299	B	0.280
C	0.667	C	0.712
D	0.017	D	0.050
E	0.117	E	0.050
F	0.453	F	0.453

Comparison of .45 caliber Acme Saloon Bullet to Typical .45 Caliber Bullet.
(Authors' Collection)

A digital microscopic examination of the bullet at 30 magnification revealed only a few striations and no pattern of lands and grooves. This was an unexplained finding. A bullet fired through a rifled barrel usually contains visible lands and grooves created from the direction of twist and numerous linear striations on the longitudinal surface of the bullet. The threads on the barrel are manufactured with the same direction of twist. Colt produces rifling with a left twist and the barrel is attached to the frame using a left thread so the torque of the bullet traveling down the barrel continually tightens the barrel to the revolver's frame.[37]

However, the number of lands and grooves and the direction of twist could not be determined for the Acme Saloon bullet. There were only a few linear striations around the circumference of the bullet. The lead bullet was shiny and there were no visible traces of lead oxide on the surface of the bullet. Lead oxide on the surface of the bullet generally appears as a white powdery substance. A bullet sealed in a glass vial in a dry climate could account for the lack of oxidation; however, the absence of lands, grooves and striations were atypical. Although, one possible explanation for the lack of class and individual characteristics may occur when a bullet is fired through a barrel of a slightly larger diameter or the bullet diameter is slightly undersized for the barrel.[38]

Examination of Cotton Fibers

A small quantity of cotton fibers were collected from the glass vial and sent to the Netherlands Forensic Institute (NFI) for forensic analysis. The NFI is a national research organization which assists local Netherlands police agencies in investigations by providing forensic examinations similar to the FBI's examinations for local police agencies in the United States. Any traces of residual material found on the surface of the cotton fibers could provide additional information about the packing process and material used to cushion the bullet in the glass vial. An Environmental Scanning Electron Microscope with Energy Dispersive X-ray (ESEM/DEX) was used to examine the cotton fibers for an elemental analysis.[39]

The ESEM/DEX instrument utilized an X-Ray to produce an electromagnetic emission spectrum to detect the elements contained in the cotton fiber sample. Twenty electron images and forty-four spectrums were collected from the cotton fibers from the sample. The fibers contained the following elements in two of the spectrums: Lead (Pb), Austenitic Stainless Steel which contained Iron (Fe), Chromium (Cr), and Nickel (Ni). Other elements detected in some of the spectrums included Sodium (Na), Silicon (Si), Sulfur (S), Chlorine (Cl) Potassium (K), Calcium (Ca), Zinc (Zn) and Titanium (Ti). Aluminum (Al) was also detected in the spectrums and was likely from the SEM sample holder. Therefore, Aluminum was not listed in Figures 1 & 2. Other elements detected in the cotton sample have possible explanations which follow.

Elements Detected on Fibers (Spectrum 1 through 10)

Spectrum	Pb	Fe	Cr	Ni	Na	Si	S	Cl	K	Ca	Zn	Ti
#1		X				X	X		X	X	X	X
#2	X							X				
#3							X	X	X	X		
#4	X											
#5		X			X		X			X	X	
#6					X	X	X		X	X		
#7	X	X						X	X			
#8	X	X	X		X	X	X	X	X	X		
#9		X	X		X		X		X	X		
#10		X	X		X	X	X	X	X	X		
Table Continued												

Figure 1 List Identification of Elements Detected on Fibers in Spectra 1-10.
(Authors' Collection)

Elements Detected on Fibers (Spectrum 11 through 22)												
Spectrum	**Pb**	**Fe**	**Cr**	**Ni**	**Na**	**Si**	**S**	**Cl**	**K**	**Ca**	**Zn**	**Ti**
#11	X	X			X			X	X	X		
#12		X							X	X		
#13	X	X	X						X	X		
#14		X	X							X		
#15		X	X	X					X	X		
#16	X	X	X		X			X	X			
#17	X	X	X	X	X	X		X	X	X		
#18		X	X	X		X				X		
#19		X	X	X		X						
#20		X	X			X	X	X	X	X	X	
#21	X	X				X			X	X		
#22		X	X						X	X		
Totals =	9	18	12	4	8	9	8	9	16	17	3	1

Figure 2 List Identification of Elements Detected on Fibers in Spectra 11-22.
(Author's Collection)

Elements Detected on Fibers (Spectrum 23 through 32)												
Spectrum	**Pb**	**Fe**	**Cr**	**Ni**	**Na**	**Si**	**S**	**Cl**	**K**	**Ca**	**Zn**	**Ti**
#23	X	X	X			X	X		X	X		
#24		X	X	X		X			X	X		
#25	X	X	X	X					X	X		
#26	X	X						X				
#27		X							X	X		
#28		X	X		X		X		X	X		
#29	X	X			X		X		X	X		
#30		X	X				X		X	X		
#31		X			X	X	X	X	X	X		
#32	X	X	X				X			X		
Table Continued												

Figure 3 List Identification of Elements Detected on Fibers in Spectra 23-32
(Author's Collection)

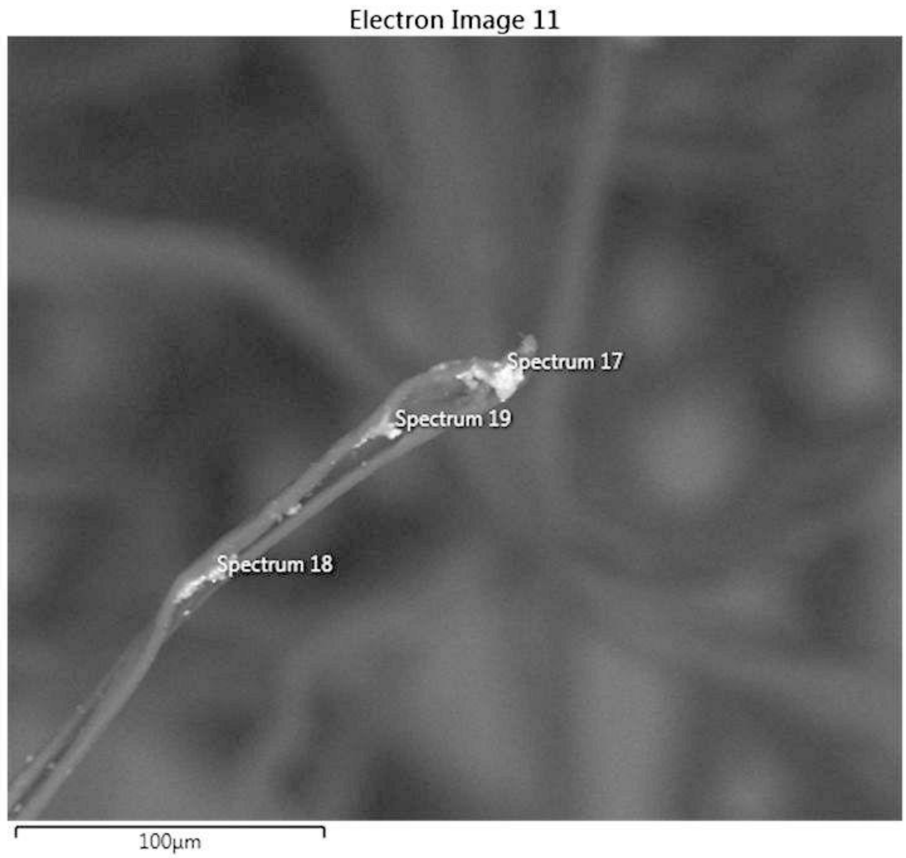
Elements Detected on Fibers (Spectrum 33 through 44)												
Spectrum	Pb	Fe	Cr	Ni	Na	Si	S	Cl	K	Ca	Zn	Ti
#33	X	X		X		X	X		X	X	X	
#34	X	X	X					X	X	X		
#35		X	X							X		
#36	X	X	X						X	X		
#37	X	X	X			X			X	X		
#38					X		X					
#39									X			
#40												
#41							X		X	X		
#42							X		X	X		
#43		X				X	X			X		
#44					X		X	X	X			
Totals =	9	16	10	3	5	6	12	4	16	17	1	0

Figure List Identification of Elements Detected on Fibers in Spectra 33-44
(Author's Collection)

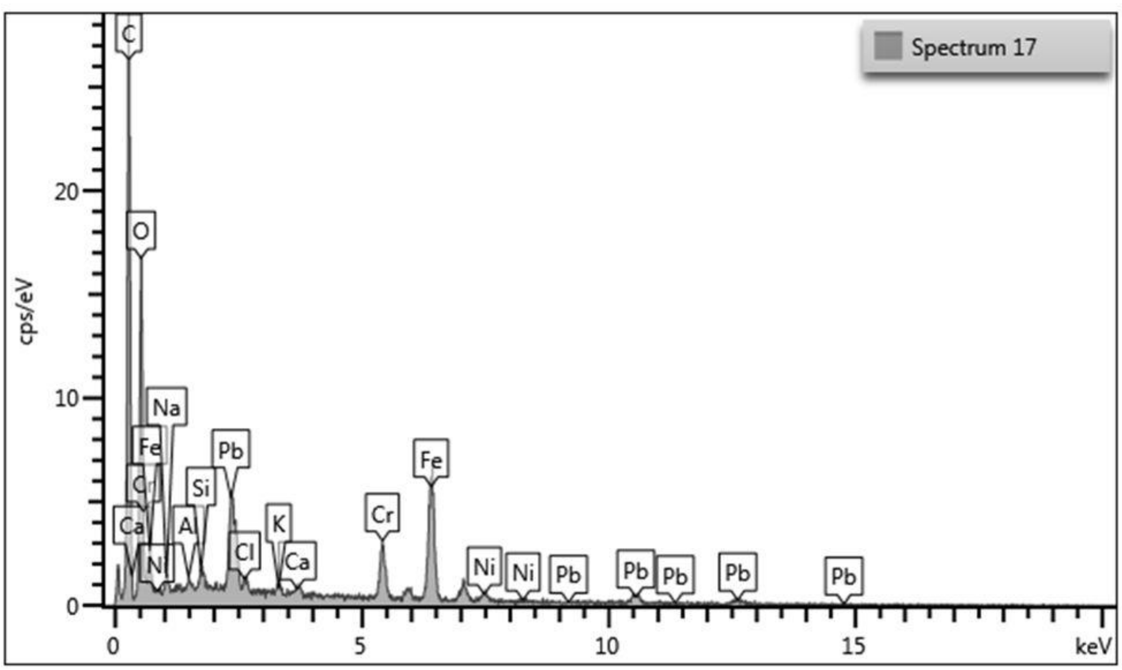
Since the Acme Saloon bullet was stored inside the vial on a ball of cotton, some of the elements detected on the cotton were from contact with the bullet and black powder also known as gunshot residue (GSR). Lead is the primary element in the bullet; however, there were other trace metals such as zinc mixed with the lead.

Black powder consists of a three-component mixture of 10% sulfur, 15% charcoal and 75% potassium nitrate.[40] This provided possible sources for the presence of sulfur and potassium. When black powder is used in a firearm, the propellant produces 44% of its original weight in gases and 56% in solid residue primarily as white smoke.[41] Some traces of iron could have been from the barrel of the revolver. Trace elements from the cartridge primer and cartridge case may also be present in the GSR. The cartridge primer consists of a pressure sensitive compound of mercury fulminate, potassium chlorate and antimony sulfide. Silicon is added to this mixture as an abrasive to ignite the pressure sensitive primer compounds when the firing pin strikes the primer. Therefore, this could account for the presence of silicon and chlorine.[42-44]

Six elements detected are illustrated in Figures 1 and 2 that have plausible origins; however, one unusual finding was the presence of austenitic stainless steel. This type of stainless steel contains iron, chromium and nickel with traces of lead.[45] Its presence on the cotton fibers are unknown and likely a contaminant from an unidentified source.



Electron Image #11 of a Cotton Fiber.
(Authors' Collection)

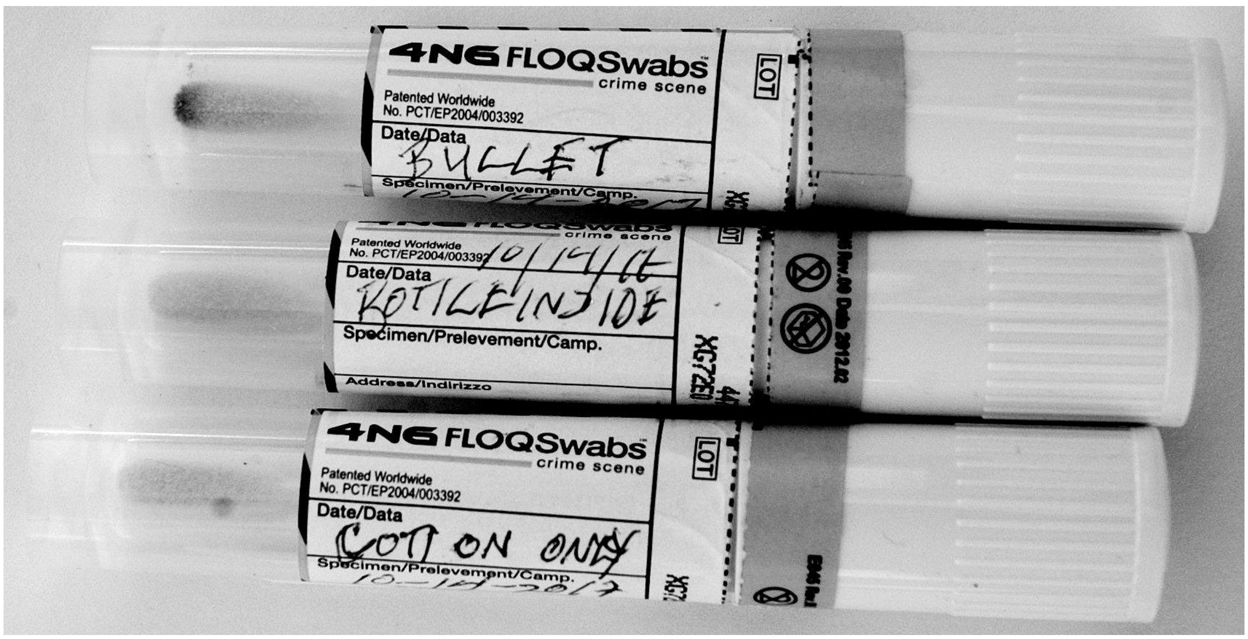


Spectra #17 from Electron Image #11 Containing Iron, Chromium and Nickel.
(Authors' Collection)

Six of the elements identified on the cotton fibers were consistent with elements present in the bullet and GSR. The presence of aluminum was likely from the sample holder. As noted previously, the presence of austenitic stainless steel was unexplained and the origin of the remaining three elements, sodium, calcium and titanium were also undetermined.

DNA Samples

The bullet was removed from the glass vial and swabbed with COPAN 4N6 FLOQSwabs™ for recovering DNA. Researchers were gloved and masked during the swabbing process to prevent cross contamination. The swabs were pre-wetted with ~15 μL of sterile water before collecting the sample. FLOQSwabs™ were used to collect the samples because they are highly absorbent swabs. The plastic applicators are coated with short Nylon® fibers arranged in a perpendicular pattern on the end of the swab. This flocking process creates a swab which maximizes sample collection.[46-47] Three samples were collected for testing, one from the surface of the bullet, one from inside the mouth of the glass vial and one from the cotton fibers.[48-49]



Samples Collected for DNA Testing.
(Authors' Collection)

In the process of physical contact with an object such as the glass vial, bullet or cotton, it is possible to deposit skin DNA which is referred to as "touch" DNA. Whether it is directly deposited or secondarily transferred, the source for "touch" DNA is from perspiration, skin oils and epithelial cells which have not lost their nuclear DNA through keratinization. The body is constantly shedding epithelial cells and it only takes a quantity of less than 100 picograms or about 16 cells to conduct DNA analysis of the sample from the donor's "touch" DNA. When multiple donors have previously handled the sample being analyzed, problems arise. Contamination and amplification of markers from multiple donors make it more difficult to accurately interpret the markers. Other variables affecting the quantity of DNA left on a surface are the physiology of the DNA donor and the environmental conditions at the time of depositing the sample or subsequent conditions to the DNA deposition.

Introduction to DNA

DNA testing and interpretation of data are complex; therefore, some DNA basics are included to better explain the DNA typing used in the John Wesley Hardin case. Much of the DNA in humans is similar; however, there are regions of DNA that vary from individual to individual. These variations are called polymorphisms. Short tandem repeat (STR) analysis is a method used to examine a specific location on a chromosome and determine if there are similarities or differences between two samples. This location on the chromosome is also referred to as the *loci* (or locus) and may consist of a motif of two to thirteen nucleotides which may be repeated hundreds of times. These STR markers are named using letters and numbers. In some instances markers are named after nearby genes, genetic disease, or biological proteins synthesized by the cell. The numeric names for these markers can provide some information about the general location within the human genome. For example, D3S1358 is the name for marker D (for DNA) on chromosome 3, present in single genome copy (S) at the 1358th locus described for that chromosome. The marker contains an STR element that is repeated many times. There are 12 prevalent variations for this marker at this location in the human population. At least 32 other more rare variations have also been characterized. These variations are called alleles. When there is a limited amount of DNA for analysis, the sample has to be amplified. In other words, artificial exact copies are generated in the lab in order to study it better. The polymerase chain reaction (PCR) is the technique used to amplify or make copies of the marker regions present in the biological sample. Low copy number (LCN) analysis is also referred to as high sensitivity analysis where additional amplification cycles are introduced in order to detect very low quantities of DNA.[50] Molecular geneticists compare the data from the markers, the number of STR repetitive DNA blocks at each marker, to determine if the two samples are likely from the same source or different sources. The frequency of each allele is known in various human populations. Therefore, the odds of a DNA profile existing at random can be calculated by a formula that incorporates the allele combination at each marker.[51-54]

For the analysis of the Hardin bullet, the DNA swabs that were collected from the surface of the bullet, from inside the mouth of the glass vial and from the cotton fibers were sent to COPAN, Brescia, Italy for testing. The analysts at COPAN used the PowerPlex® Fusion 6C System, a kit manufactured by Promega, to type or generate a profile from the DNA samples. The kit tests for twenty-seven markers including those used for personal identification in the FBI's Combined DNA Index System (CODIS), as well as other markers required by countries in the European Union (EU). The analysts also used the Yfiler® Plus System manufactured by Thermo Fisher Scientific which queries a set of twenty-five STR markers present on the male Y-chromosome (Y-STRs), thereby generating a Y-chromosome profile for the male contributor. Both the PowerPlex® Fusion 6C and the Yfiler® system rely on the polymerase chain reaction amplification process.[55-58]

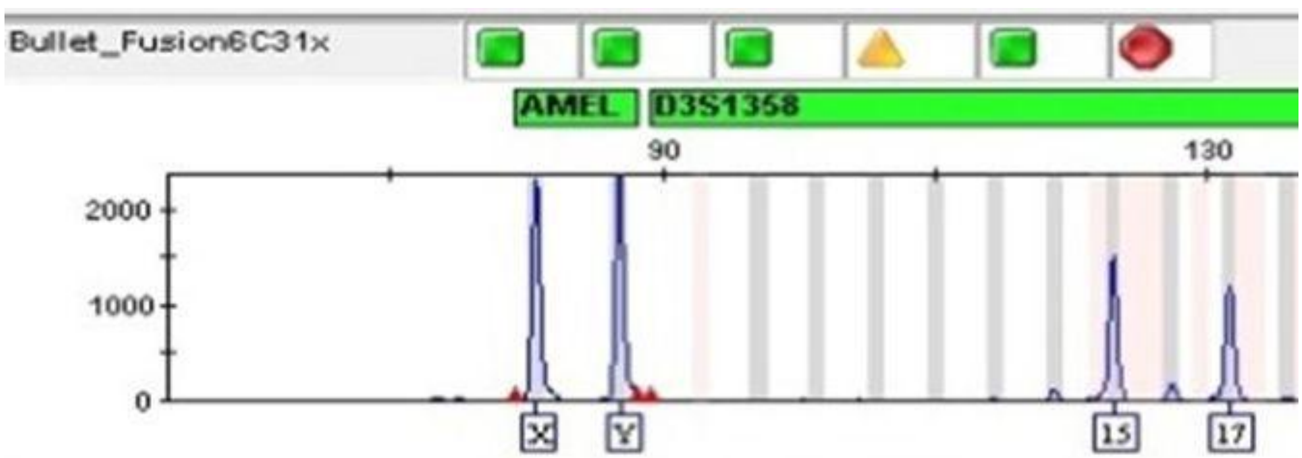
Each sample was run twice to establish some degree of confidence in the marker yield. The height of the peaks in the electropherogram diagram is measured in relative fluorescence units (RFUs) and reflects the amount of DNA detected via amplification at each marker. The number under the peak is the allelic (STR repeat) number. For instance, the DNA profile generated from the bullet shows results at the D3S13 marker; the donor had two peaks, a 15 and 17. One of the alleles, either 15 or 17 came from the father and one came from the mother. When there is only one peak for a marker, as in the case at the D2S441 marker, this indicates the mother and father donated the same numeric allele 14 for the DNA on the bullet. When there are three peaks at a marker as seen at marker D8S1179, alleles 11, 13 and 14, DNA is present from someone other than the mother and father. Depending on the data seen in the entire profile, it is possible that three peaks or more could be an admixture from three or more donors. Laboratory policy sets a threshold number for the amount of DNA needed for the analysis and other quality criteria to determine the reliability and interpretation of the results.

Once testing is complete and the raw data is generated, the analyst uses specialized computer software to create a visual chart for each profile known as electropherograms or e-grams and evaluates the quality and interpretability of the data in preparation for profile comparisons. The software color codes each marker based on pre-set quality parameters that the analyst can control, such as, the peak height in RFUs, any allele height imbalances as well as presence of potential mixtures and signal artifacts. The marker is labeled in green if all quality measures are met, whereas the marker will be flagged as yellow or red if there are questionable quality values that the analyst should take into consideration.

The DNA markers recovered on the bottle and cotton generally reflect the quantity of DNA expected from "touch" sources. The quantitation results also show some level of DNA degradation. The DNA profiles indicate multiple donors which is not unusual since numerous individuals may have touched or handled the glass vial and cotton during the past one hundred and twenty-three years. However, the quantity of DNA recovered from the Acme Saloon bullet is approximately five times higher than the DNA quantity recovered from the bottle and the cotton.

It is noteworthy that a full DNA profile was recovered from the bullet. Both testing kits revealed nearly a complete male DNA profile with potentially a second low-level contributor. The first marker on the following list of markers is the AMEL marker. This marker has no allelic number; it tests for the presence of the X and Y chromosomes and is used to determine the gender of the DNA donor. The AMEL marker indicates the DNA on the bullet is from a male donor. The presence of male DNA is also revealed by an allelic peak present at the Y-Chromosome marker DYS391. The other markers tested by the PowerPlex® Fusion 6C kit are: D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, THO1, VWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, SE33, D22S1045, DYS391, FGA, DYS576, and DYS570.

The markers tested by the Yfiler® kit are: DYS3891, DYS635, DYS38911, DYS627, DYS460, DYS458, DYS19, YGATAH4, DyS448, DYS 456, DYS390, DYS438, DYS392, DYS518, DYS437, DYS385, DYS449, DYS393, DYS439, DYS481, DYF38751, and DYS533. Whether this profile originated from a rich source of nucleated DNA such as from blood is unknown.



Electropherogram Peaks for AMEL and D3S1358 Markers.
(Authors' Collection)

In conclusion, the bullet measurements, SEM analysis of cotton fibers and DNA tests contributed to previously unknown information about the bullet Stevens collected from Hardin's death scene in the Acme Saloon in 1895. Even though some news articles reported the revolver Selman fired was a .44 caliber, the measurements and weight of the Acme Saloon bullet were more consistent with .45 caliber vintage bullet weights and measurements. Additionally, one unusual finding from the microscopic examination of the Acme Saloon bullet was the lack of lands and grooves as well as linear striations so perhaps the bullet was fired through a barrel of a slightly larger diameter or the bullet diameter was slightly undersized for the barrel. Also, SEM elemental analysis of the cotton that cushioned the bullet in the vial was consistent with some elements identified in GSR. The implication of the presence of these elements suggests the bullet was a fired bullet and not a fabricated fired bullet. However, the only unexpected and unexplained material on the cotton fibers was the presence of austenitic stainless steel. Stainless steel had not been invented at the time of Hardin's death. While the DNA on the inside of the glass vial and cotton were an admixture from more than one individual, the male DNA on the bullet was noteworthy and may be suitable for familial DNA searching. Even though John Wesley Hardin does not have any known living male descendants, perhaps his brothers have known living male descendants. In particular, a comparison between the Y-STR profiles from known male Hardin family descendants could be compared to the Y-STR DNA profile found on the bullet to investigate a possible common male lineage. The male Y-chromosome is inherited paternally and therefore, descendants from a common male ancestor are expected to have very similar, if not the same, Y-STR profiles. Consequently, the researchers will continue to pursue identifying the DNA found on the Acme Saloon bullet.

Acknowledgements

First and foremost, the authors would like to thank Bill Koch for granting the researchers access to the Acme Saloon bullet for forensic examination. We also appreciate Mr. Koch's staff, Natasha Khandekar, Tom and Mary Glor, Tracie Edling, and Xiomi Murray for their assistance. Mike Cox, Wild West History Association Journal editor, provided valuable assistance during the bullet examination and the researchers are genuinely grateful to him for his help. Furthermore, the authors greatly appreciate the Netherlands Forensic Institute, Den Haag, for participating in this research. Finally, we are grateful to Santina Casticiano and Michele Rosse with COPAN, for DNA analysis in this case, contributions to forensic research and continued support of the researchers.

Endnotes:

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[7] John Lachuk, *Guns and Ammo Guide to: Guns of the Gunfighters, John Wesley Hardin* (Los Angeles, CA: Peterson Publishing Company, 1975) pp. 79-84.

[8] *The Estate of Richard C. Marohn, M.D.* (auction catalog) (San Francisco, CA: Butterfield and Butterfield, 1996) p. 126.

[9] Authors' Note: Different numbers of killings are reported by different news sources; however, according to the newspaper article, "John Selman Shot," *The Richmond Item*, (Richmond, Indiana) 6 April 1896, p. 2, Selman killed twenty men in the State of Texas.

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[12] John Wesley Hardin Collection, MS 030, C. L. Sonnichsen Special Collections Dept., The University of Texas at El Paso Library, D. Storms' Handwritten Notes, 20 August 1895.

[13] W. C. Jameson, *Unsolved Mysteries of the Old West* (New York: Taylor Trade Publishing, 2013) p. 126.

[14] Authors' Note: The Colt Peacemaker had several name changes during early production; the first factory designation was the "New Model Army Metallic Cartridge Revolving Pistol." (Dennis Adler, *Cold Single Action: From Pattersons to Peacemakers* (New

York, NY: Chartwell Books, Inc., 2007, p. 197) It was also called the Colt Model 1872. In 1873 the model was referred to as the Peacemaker, the Frontier Six Shooter and SAA for Single Action Army. Charles Edward Chapel, *Guns of the Old West: An Illustrated Guide* (Mineola, NY: Dover Publications, Inc., 2002) p. 232.

[15] Lachuk, *op. cit*., pp. 79-84.

[16] Authors' Note: The revolver used to shoot Hardin is in Dr. James H. Earle's collection of Western memorabilia, Bryan, Texas. Dr. Earle is Professor Emeritus, Civil Engineering, Texas A&M University.

[17] *The Estate of Richard C. Marohn, M.D*., *op. cit.* p. 126.

[18] Authors' Note: A reprint of the *El Paso Herald* (El Paso, Texas)·20 August 1895 article in the *El Paso Herald* (El Paso, Texas)·19 August 1909, "John Wesley Hardin Killed in El Paso 14 Year Ago."

[19]·Authors' Note: According to Brian Kevin, *Gun History & Development* (Minneapolis, MN: Abdo Publishing Company, 2012) p. 16, claims Austrian gunsmith, Gaspard Kollner is created with the development of rifling in the 1400s.

[20] Authors' Note: According to Thomas Francis Fremantle, *The Book of the Rifle*, (New York: Longmans, Green and Co., 1901) p. 5 claims the rifling process was attributed to Augustus Kollner of Nuremburg in 1550.

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[27] A Noted Outlaw Killed, *op. cit.,* p. 5.

[28] "A Frontier Incident," *Pittsburgh Daily Post* (Pittsburgh, Pennsylvania) 23 August 1895,·p. 4.

[29] Frank C. Barnes, *Cartridges of the World*, 9th ed. (Iola, Wisconsin: Krause Publications, Inc., 2000), p.285.

[30] Authors' Note: According to Walter Otheman Snelling and Christian George Storm, *The Analysis of Black Powder and Dynamite* (Washington D.C.: Government Printing Office 1913), pp. 66-67, 2F black powder refers to the approximate diameter of the powder grains. The 2 F black powder grains will pass through a screen which has 14/64th diameter holes and collect in a screen which has 7/64th diameter holes.

[31] Edward Matunas, *American Ammunition and Ballistics*, (Tulsa Oklahoma: Winchester Press, 1979), p. 92.

[32] Authors' Note: A joule is a unit of energy in the International System of Units. A joule is equal to the kinetic energy of a kilogram mass moving at the speed of one meter per second.

[33] Werner U. Spitz and Daniel J. Spitz, eds., *Medicolegal Investigation of Death: Guidelines for the Application of Pathology to Crime Investigation*, 4th ed. (Springfield, Illinois: Charles C. Thomas Publisher, 2005), p. 690.

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[35] Arnold Lorentz Ahnfeldt, John Boyd Coates, and Robert S. Anderson, *Medical Department of the United States Army in World War II* (Washington, DC: Office of the Surgeon General, Department of the Army, 1962) n.p.

[36] V. J. DiMaio, "Penetration and Perforation of Skin by Bullets and Missiles. A Review of the Literature," *American Journal Forensic Medicine and Pathology* 2, no. 2 (1981) pp. 107-110.

[37] Authors' Note: According to Robert A. Rinker, *Understanding Firearm Ballistics*, 4th ed. (Chelmsford, England: Mulberry House Publishing, 2002) p.128, U.S. firearms makers that use a right twist in the barrel use a right thread to attach the barrel to the frame so the torque tightens the barrel to the frame in the same manner as Colt firearms with a left twist.

[38] Authors' Note: According to T. Holloway, *A Guide to Handgun Cartridges: A Reference for Common Calibers* (Morrisville, NC: Lulu.com, 2015) p. 141, the .45 Colt, also called .45 Long Colt (.45 LC) is a black powder handgun cartridge dating to 1872. This cartridge was adopted by the US Army in 1873 and was the official military cartridge for the Colt Single Action Army revolver for nineteen years. It is referred to as the LC to prevent confusion with shorter cartridges, the .45 Schofield and .45 S&W.
[39] Authors' Note: The type of electron microscope used in the analysis was an FEI Quanta 400 Environmental Scanning Electron Microscope (ESEM). Operating conditions of the microscope varied from an accelerating voltage of 15-25 kV, depending on particle characteristics and analytical goals. For chemical analysis, the ESEM was equipped with an energy dispersive x-ray microanalysis system, using an Oxford Instrument Si(Li) detector in combination with Oxford INCA GSR-software. Most analyses were performed in the 'low-vacuum' (p = 10-20 Pa) mode of the SEM.
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[41] Gerald R. Styers, "The History of Black Powder," *AFTE Journal*, 19, no. 4 (1987), pp. 443-446.
[42] G. Martin, *Industrial and Manufacturing Chemistry Organic: A Practical Treatise* (New York, NY: D. Appleton and Company, 1913), pp. 637-638.
[43] *Treatise on Ammunition: Printed for His Majesty's Stationery Office*, 7th ed. (London: Harrison and Sons Printers, 1902), pp. 1-2.
[44] T. L. Davis, *The Chemistry of Powder and Explosives* (Hollywood, California: Angriff Press, 1943), pp. 454-455.
[45] J. F. Sewell, "Stainless Steel and Armour Plate," *The Guardian* (Greater London, England), 9 October 1958, p. 10.
[46] Authors' Note: COPAN, Italy patented a Swab for Collecting Biological Specimens. The US Patents are # 8,114,027, #8,317,728, #8,979,784, #9,011,358, #9,173,779, European Patent #1608268, Canadian Patent #2515205, Japanese Patent # 2007-523663, Australian Patent #2004226798, New Zealand Patent #541560, and Chinese Patent #101103931.
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[53] Leonard Klevan and Lisa Lane Schade, "Identifying Degraded DNA," *Forensic Magazine* (February/March 2007), n.p.
[54] Jennifer Clay, "Y-STRs - The Proof is in the Results," *Forensic Magazine* (April/May 2007), n.p.
[55] Ana Boavida, Vanessa Bogas, Lisa Sampaio, Nair Gouveia, Maria J. Porto and Francisco Corte-Real, "PowerPlex® fusion 6C System: Internal Validation Study," *Forensic Sciences Research*, (Abingdon, UK: Taylor & Francis Group, 2018) DOI: 10.1080/20961790.2018.1430471.
[56] Authors' Note: The Original CODIS Core Loci, required from October 1998 until December 31, 2016, included the following 13 loci. They were CSF1PO, FGA, THO1, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11. Effective January 1, 2017, the CODIS Core Loci was increased to 20 loci. The additional loci added to the original 13 loci were D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433, and D22S1045.

[57] Alice Squassina, Michele Rosso, Vania Rigon, and Elisa Piovanelli, (Copan Italia S.p.A, Brescia, Italy), "How COPAN Adjusted to the New International Standard ISO 18385: In Order to Minimize the Risks of Detectable Human DNA Contamination in Its Forensic Products," Poster Presentation, 28th International Symposium on Human Identification, Seattle, WA, October 2-5, 2017.

[58] Authors' Note: The markers used in the FBI database are only used for personal identification. They have no biomedical relevance for disease.

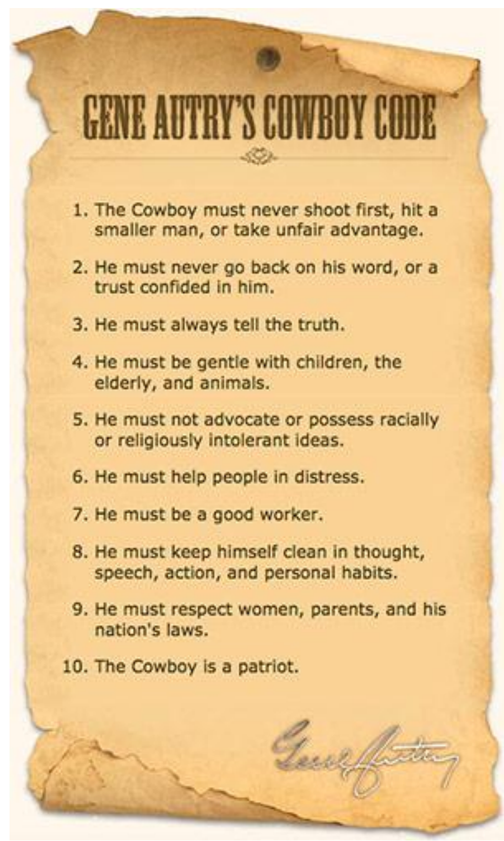
At the recent Aspen Institute on "The American West: The History, The Values, The Struggle, & The Legacy of the 19th Century," an evening panel session "Values in Conflict: The Code of the West," truly struggled to define and enumerate the qualities of such a code.

The panel's specific assignment revolved around this statement and the concluding question: "The story of the American West is a clash of cultures and values. In a series of brief statements followed by moderated discussion, leading scholars of the American West expose a broad range of perspectives that challenge our understanding of the code of the West. What was emerging in the American character and what separates the reality from the myth?"

Obviously, the code may consist of many things: qualities, standards, guidelines, life purposes. The idea was popularized in the 1940s and 50s by a fine group of cowboy movie stars: Hop-a-Long Cassidy, Roy Rogers, Gene Autry, and others such as the Texas Rangers, John Wayne and the memorable "Lone Ranger and Tonto." Actors Clayton Moore and Jay Silverheels, were said to have tried their best to live by this code. Moore publically said he strove in his personal life to take "The Lone Ranger Creed" to heart.

Some historians say that Zane Grey was the first one to write about the "code." He wrote a book with the title "Code of the West" that became a movie of the same name in 1925 starring Owen Moore and Constance Bennett and one in 1947 starring James Warren and Raymond Burr. Other books and movies using the title have appeared as have written codes by numerous lesser-known personalities, such as a documentary produced in 2012 by Rebecca Richman Cohen dealing with the use of legalized medical marijuana in Montana.

The scholars on the Aspen Institute panel struggled mightily, and finally settled, somewhat, on Gene Autry's version of the code:


GENE AUTRY'S COWBOY CODE

1. The Cowboy must never shoot first, hit a smaller man, or take unfair advantage.
2. He must never go back on his word, or a trust confided in him.
3. He must always tell the truth.
4. He must be gentle with children, the elderly, and animals.
5. He must not advocate or possess racially or religiously intolerant ideas.
6. He must help people in distress.
7. He must be a good worker.
8. He must keep himself clean in thought, speech, action, and personal habits.
9. He must respect women, parents, and his nation's laws.
10. The Cowboy is a patriot.

Gene Autry

Are these qualities yet the qualities of the West? Does Autry's code of the West stand the ravages of time?

All WWHA members are invited to submit a letter to the editor relating your vision and understanding of the "Code of the West." We will publish a selection of letters in the December issue of our *Journal*.