

Linking the forensic biology classroom to the courtroom

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Abstract: The concept of clear communication and truth in testimony is examined in the forensic biology classroom by introducing undergraduate students to the American Society of Crime Laboratory Directors (ASCLD) guiding principles and then having an evidentiary hearing transcript evaluated for key concepts from an adjudicated post-conviction case where blood pattern analysis, DNA test results and legal definitions are considered carefully. The intrigue in this case develops after a blood pattern expert is identified as fraudulent and nonstandard science is applied after poor evidence collection and storage practices. The goal with this exercise is to examine each word of the transcript with pre-identified court issues to determine if students believe the concept from the transcript to be true. A comparison to current best practice recommendations is also provided in student discussions and demonstrations to aid them in evaluating the changes in forensic science practices from the pre-DNA to post-DNA era that may often be encountered in post-conviction review work.

Keywords: ASCLD guiding principles, courtroom testimony, forensic biology, DNA, blood pattern analysis (BPA), evidence collection

Introduction

The American Society of Crime Laboratory Directors (ASCLD) has set forth a set of guiding principles for the forensic scientist that includes expectations for professionalism, clear communication, and proficiency (1). Learning about the guiding principles and considering professional work obligations and ethics is part of the education of the forensic scientist in the course curriculum or the workplace. The purpose of this classroom exercise is to provide training and discussion in evaluating best practice and clear communication strategies for entry into the forensic biology workplace. The guiding principles were written for forensic scientists and laboratory management. These principles have been adopted by the ASCLD/LAB Board of Directors as guides and for use in training. Some key aspects identified under the guiding principles include professionalism (e.g. conduct fair and impartial examinations; render conclusions that are within expert's area of expertise), competency and proficiency (e.g. proper training and competence; care in treatment of samples to avoid tampering, adulteration, loss or unnecessary consumption), clear communications (e.g. accurate representation of expert's education, training, experience and area of expertise; be able to support sound scientific techniques).

Blood identification and blood pattern analysis methods require skill and professional training. Chemical tests are typically used to presumptively screen and then potentially confirm by immunology the presence of human blood on a garment. While blood identification is part of many program curriculums, many forensic science

programs shy away from pattern interpretation until students have examined the effects of gravity and force on blood droplet shape. The traditional methods for pattern analysis training include dropping known volumes of blood from various heights and at different angles to examine the effects on the shape of a blood droplet. Often, though, clothing evidence is received in forensic laboratories in a variety of conditions (e.g., torn, ripped, worn, folded/unfolded, dirty) and one good question that might arise for stain patterns is whether the stain was original to the crime scene or was from blood transfer during folding and storage? For the student (in preparation for the case evaluation), several good articles discuss blood identification methods (2-3). Additional articles discuss the challenges of blood pattern interpretation and the determination of age of bloodstains (4-8).

Described here is an alternate approach to the method of learning blood identification techniques used in the forensic laboratory where an evidentiary hearing transcript is dissected for methods, best practice, and scientific accuracy. The case details and approach are described in the methods section below. Students also attend the forensic biology laboratory and perform microscopy and presumptive blood screening exercises using phenolphthalein and ortho-tolidine to establish the authenticity of the transcript statements using their own hands. This provides a framework for examining science and technology junctures where older laboratory practices may have been supplanted with newer techniques and do not always yield the same result. While learning about the scientific techniques used in the laboratory, it is important to understand them at a deeper level when one

must go to the courtroom to explain the technique in detail and a written record or transcript is maintained.

A puzzle that is difficult or impossible to solve is called a conundrum. This case is an example of a conundrum. As a training exercise in forensic biology and its applications to post-conviction testing, students are asked to examine the information posted to multiple website sources to review the background on this double homicide case. The case (*State of North Carolina v. George Goode, Jr.*) was a particularly bloody double homicide with a landlord and landlord's wife brutally stabbed to death and four individuals purported to be involved. One of these individuals, George Goode Jr. had no human blood identified on his clothing at the time of the crime in 1992 (9). The question at hand was if no human blood was identified on him, was it true he was a witness as he claimed rather than a participant? Without video surveillance or eyewitness testimony, only possible case circumstances can be determined, and the presence or absence of blood became a critical factor in establishing Mr. Goode Jr.'s proximity to the homicides. There are strong opinions in this case by counsel on both sides starting from the original trial all the way through a very long appeal process. When DNA technology became available for post-conviction testing, it was thought that DNA testing would result in a possible exoneration.

One of the key issues with this case revolved around whether George Goode Jr. was an active assailant or an innocent bystander. Three other individuals were convicted in this case as being active participants and they did have substantial amounts of blood on their clothing. George Goode Jr., however, did not. Leon and Margaret Batten were viciously stabbed to death and on November 19, 1993, George Goode Jr. was found guilty and given the death penalty even though he testified that he was merely present as a bystander. Leon Batten was the landlord where George Goode Jr. and his wife lived. George Goode Jr.'s lack of blood evidence played heavily in the decision to re-examine his clothing for post-conviction DNA analysis. A motion was filed for appropriate relief (MAR) where a judge was asked if post-conviction DNA testing could be performed to clarify the issue. This is a statutory mechanism to challenge a problematic conviction. The judge has the full discretion to hear the motion. MAR hearings are typically granted for a variety of reasons: (1) the defendant did not understand the full impact of a guilty plea, (2) the court misapplied the law, (3) the evidence did not support the jury finding, (4) new evidence or technology to analyze the evidence has become available, (5) the defense counsel did not provide adequate representation or (6) a new law has been made that retroactively affects the conviction

Concept 1: Can microscopy be used effectively as a screening method to determine a substance and the manner in which it was applied to clothing?

The following are quotes from a transcript of the MAR proceedings regarding the examination of clothing evidence for post-conviction DNA testing results taken in the General Court of Justice, Superior Court Division, Johnston County, North Carolina at the September 13, 2004; Special Criminal Session (File NOS. 92-CRS-2661-6292-CRS-2569, 2570; Volume 10, pages 1616-1819).

The Coveralls. Serologist Bissette testimony "I made a visual observation of those coveralls, and I did a phenolphthalein test; and I found that test to be negative" (MAR transcript page 1730 lines 2-4.). This statement was made in reference to Bissette's original serological evidence examination in 1992. Bissette found "that the garment was soiled and there were some grease-like stains" (MAR transcript page 1731 lines 2-3). No human blood was identified at the time of the crime.

A phenolphthalein test, also called the Kastle-Meyer reagent test, is a presumptive test for screening for possible blood. A swab is collected from a blood-like stain and the test reagents (reagent 1: phenolphthalein; reagent 2: 3% hydrogen peroxide) are added consecutively and the pink color change read as an indicator of a positive test for possible blood. This test is for screening of evidence and does not confirm the presence of human hemoglobin, a protein whose presence is required for the formal identification of human blood. It could turn positive with animal blood and some plant or soil components. Since the presumptive blood identification screening test was negative, no further confirmatory testing for human blood was performed on George Goode Jr.'s coveralls in 1992. This would have been considered standard and best forensic practice at the time.

In 2004, a hearing was held to evaluate the process and results of the re-testing of the clothing evidence in post-conviction DNA analysis. In this hearing, Bendure describes his role using microscopy in selecting areas of the clothing to use for the DNA test but never performs a human blood identification step to answer the critical question of whether the stains were grease or human blood. He also attempts to address the question of whether the blood-like stains now visible on the coveralls are from the time of the crime or from contamination events during storage based on a visual assessment of adherent or absorbed stains.

Microscopist Bendure testimony "We do proficiency tests for identification purposes. I've not performed any identification here. I've just described the material that appeared to be something to me. I never said the samples were blood. So, therefore, I've done no blood identification. Therefore, I do no proficiencies on blood identification. I'm not a serologist or a DNA person. I just

mainly look at materials because I know what they look like. I went in and isolated the material and provided it to the DNA unit (MAR hearing page 1677 lines 17–25-page 1678 line 1). This part of the testimony relates to his skill set and the guiding principles of professionalism, competency and clear communication for determining a substance and the timing of deposit using microscopy.

In response to whether Bendure had been proficiency tested to recognize blood or blood-like substances, Bendure's testimony was "so my opinions about the state of wetness or blood being absorbed in the yarn is not really what I would call expert opinion. It's observation of my experience and common sense" (MAR hearing page 1678 lines 1-12).

And "I just know what grease stains look like" (MAR hearing page 1679 line 13)

And "I've never been (proficiency) tested, but I know what paint looks like" (MAR hearing page 1679 lines 18-19)

And "And I know what ketchup looks like too" (MAR hearing page 1679 line 2)

Concept 2: Can possible blood be invisible and under what circumstances would this be true?

The Boots. Another feature of this case involves the term "invisible blood." How can an expert testify to something they cannot see? Is it even possible to find circumstances where this could be true? How would this relate to evidence in this case?

Excerpt: "STATE of North Carolina, v. George Earl GOODE, Jr. No. 10A94 (1).

"State Bureau of Investigation Special Agent Duane Deaver, who was proffered as an expert in the field of forensic serology and bloodstain pattern interpretation, testified that although he found no visible bloodstain located on defendant's boots, a chemical test indicated the presence of (presumptive) blood, the type of which could not be determined. Agent Deaver did not detect any visible bloodstains on defendant's coveralls, hat, or boxer shorts. It was Agent Deaver's opinion that the absence of blood on any of defendant's clothing had no exculpatory effect.

Defendant next assigns as error the trial court's admission of SBI Special Agent Deaver's testimony concerning a microscopic quantity of blood on the top leather portion of defendant's left boot. Other than revealing the presence of this "invisible" blood, Agent Deaver could draw no further conclusions as to the type or source of the minute quantity of blood he found. The

invisible bloodstain could not be tested further to establish if it was human blood".

Given these two positions stated above regarding nonvisible blood that could not be confirmed as coming from a human source, would it be the more reasonable to assume "the blood" was neither tested nor proven to be human blood? This point, again, goes toward ascertaining proximity to the victims and the issue of George Goode Jr. not having any identified human blood on his clothing or shoes at the time of the crime. Has the presumptive test for blood identification met the legal standard for testimony of blood?

Methods

Exercise 1

As part of the training in using presumptive and confirmatory forensic identification tests for blood and human blood, I discuss this case to show the value in being able to be certain the substance is human blood and be able to comfortably testify to the identification of the substance. Otherwise, the term "blood-like substance" must be used in the courtroom. Proper training and experience along with proficiency testing translates to competence in the laboratory and courtroom.

Students are asked to examine 5mm x 5mm squares of white cotton cloth under stereomicroscopes (4-10x magnification power) that have had red-brown substances applied that are then air dried. This is not a proficiency test per se, but students are asked to review all the samples and asked to rank them for which they would consider might be blood (human or animal) based on the microscopic appearance. Samples include human blood, pig blood, barbeque sauce, red lipstick, red nail polish, red-brown house paint, tea stains, potassium permanganate and ketchup. This exercise is to illustrate to the student that some substances can appear blood-like and with careful observation, a student may be able to narrow down the likely stains to the human and animal bloodstains, but presumptive and confirmatory human blood testing would be required to distinguish between those two options scientifically and legally for reports and testimony. Bendure was clear about his competency in his testimony stating that he was trained only in microscopy.

The screening for possible blood by microscopy is useful to orient the student in basic microscopy skills. As a refresher, the microscope is examined for all its component parts and focus, and photography is reviewed. The 5mm x 5mm test squares are not labeled but are provided as unknowns to the students who then sort them into options of possible blood, possible food stains, possible paints or other as categories. Students are asked to write a detailed description of the substance in their

own words (dry, flaky, absorbed, uniform in color, smooth, etc.) on an evidentiary worksheet as well as sketch the weave pattern of the fabric. On completion of the exercise, students are given the correct answer to the source of each unknown substance.

This exercise is useful for a discussion on the use of adherent versus dried flaky blood as an observation to support a crime scene theory. Bendure used microscopy and adherent blood to threads to suggest that Bissette was incorrect in 1992 and the grease stains were blood-like stains that she incorrectly identified. He theorized that if the stains appeared absorbent, then the “blood” had been applied wet, therefore, it must have been there at the time of the crime. There is very little peer-reviewed scientific literature on this topic and thus the judge could only rely on the microscopist's opinion for guidance. It would have been helpful to know what “blood” looked like many years later when co-mingled clothing evidence was stored in humid conditions at room temperature.

Exercise 2

As a class, I ask students if any can identify a possible circumstance where blood could be considered "invisible" while acknowledging the fact that it could not be analyzed further. Students are given a series of small beakers with neat pig blood (obtained from a butcher shop) and a series of dilutions of the animal blood in water (1:10; 1:50; 1:100; 1:500; 1:1000; 1:10,000; 1:100,000 and 1:1,000,000) to test with phenolphthalein and ortho-tolidine for establishing a sensitivity of detection of possible blood.

Each student is given a clean ceramic plate with wells and asked to place a single drop of each dilution into separate wells to create two separate experiments for each test reagent. To each sample of one plate, one drop of test reagent phenolphthalein is added, and a color change is noted after 10 seconds. If no color change, 1 drop of 3% hydrogen peroxide is added and any color change to pink is recorded after 10 seconds. The timing of this test is important as false positives can appear if the results are read after the 10 seconds. The second plate is set up the same way except the test reagent is ortho-tolidine which when positive results in a blue color change. The test method is performed the same way as for phenolphthalein. A class discussion is held on (a) which test reagent is more sensitive based on the color detection of weak diluted samples and (b) the effect of clothing color and dyes on the ability to reliably interpret presumptive blood detection results. At some point, students realize that extremely dilute blood samples could be difficult to visualize but still have trace amounts detectable by chemical presumptive test methods.

Results

Most students can sort possible bloodstains into the correct category for Exercise 1. An example of a human bloodstain analyzed and documented by photo microscopy is shown in **FIGURE 1**. This stain was formed by a direct deposit on the surface of the absorbent fabric, and it is evident that the blood was wicked into the fabric and then dried.



FIGURE 1 Human bloodstain on cotton fabric examined at 40x magnification with a Fisher brand stereomicroscope. Photograph is courtesy of Sophie Hryzan, University of New Haven.

Presumptive blood detection reagents all differ in their ability to detect human blood especially when diluted as evidenced by the results from *Exercise 2*. After performing the testing for presumptive identification of blood, students can detect a limitation based on the sensitivity of detection for the two reagents phenolphthalein and ortho-tolidine. A phenolphthalein test has a limit of detection of 1:500 dilution beyond which blood could be invisible in this example (**FIGURE 2**).

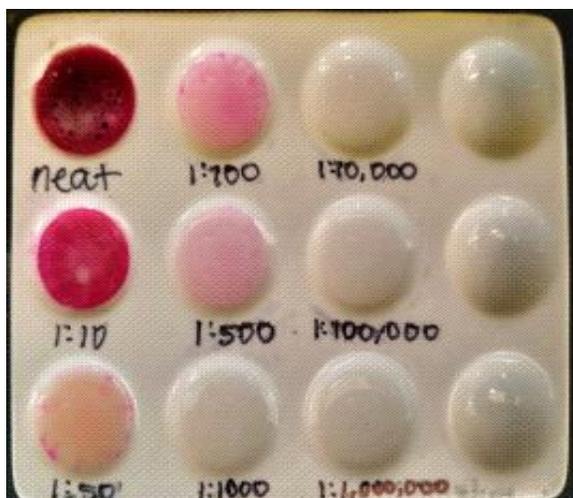


FIGURE 2 Test results for the phenolphthalein presumptive test for blood identification. This test is not human-specific and can detect animal blood. The blood: water dilution is labeled below each sample well in the spot plate. The neat sample is undiluted animal blood. Phenolphthalein is a detection test for heme in blood and a pink color indicates a positive result. The shade of pink is loosely correlated to the quantity of heme in the sample.

The same sensitivity of detection concept holds true for the ortho-tolidine presumptive blood identification test with a limit of detection at 1:1000 (**FIGURE 3**). In this example, ortho-tolidine was the more sensitive detection reagent when compared to phenolphthalein but that may be a simple matter of greater contrast with the dark blue color for the positive test result.

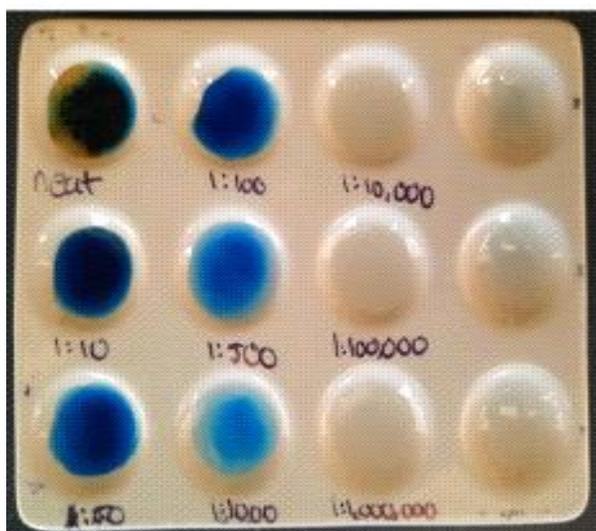


FIGURE 3 Test results for the ortho-tolidine presumptive test for blood identification. This test is not human-

specific and can detect animal blood. The blood: water dilution is labeled below each sample well in the spot plate. The neat sample is undiluted animal blood. Ortho-tolidine is a detection test for heme in blood and a blue color indicates a positive result. The shade of blue is loosely correlated to the quantity of heme in the sample. This example shows that in comparison to phenolphthalein, ortho-tolidine is the more sensitive detection reagent.

This exercise should reinforce the need for students and analysts in forensic science to know the reagent and method limitations. A good point to be made with presumptive test methods is any plant peroxidase and many heavy metals can yield a false positive reaction. The stains in question on the coveralls and boot are in locations where an individual walking in a ditch or a field of grass may have acquired plant peroxidases as stains on clothing. It also shows that to testify to a substance as being human blood, a proper confirmatory test would need to be performed to identify human hemoglobin. In the situation of the boot, the sample was consumed in the preliminary presumptive test and could not be further confirmed as being from a human source. This finding should also prompt a discussion on forensic laboratory evidence consumption policies and the need to retain evidence, when possible, for replicate or post-conviction testing.

Students show competency with microscopy and analysis of magnified bloodstains in this laboratory. Most can distinguish between blood (animal, human) and the other red-brown substances applied on the cloth samples. Students also are appreciative of the fact that highly dilute blood samples can be invisible to the naked eye such as those that might be encountered in bathtubs or sinks where blood has been cleaned up at a crime scene. The blood dilution series experiment reinforces the sensitivity of detection limits of presumptive blood detection reagents.

Discussion and Conclusion

For classroom discussion, I do acknowledge that without chemical testing for confirmation of human blood, it is difficult to know what was tested after 1992 from the George Goode Jr. clothing samples in the post-conviction analysis. Was it grease (with contaminating blood-like stains from comingled storage) as defense counsel claimed? Or was it human blood that was not correctly identified in the original biological examination in 1992? The lack of confirmatory testing for human blood (this step was by-passed by the laboratory in the post-conviction DNA testing in lieu of microscopic analysis) and poor storage conditions after trial further complicate and hamper the modern era analyst from definitively evaluating the old evidence. The evidence

after the original trial was stored unwrapped in a large plastic bin in the bottom of a courthouse and comingled with other unwrapped items including two open and empty vials of blood from the victims and a bloody tailgate used to transport both of their blood-soaked bodies for emergency services. Due to these unfortunate circumstances and poor forensic storage practices after the original trial, the evidence had been potentially contaminated, and any DNA results would be perplexing. The use of new DNA technology to determine the source of the grease/blood patterns that remained on the clothing was thought to be useful but may not have fully addressed the issue due to the poor evidence storage conditions. It did confirm the presence of Leon and Margaret Batten's DNA as well as some additional DNA mixtures.

This laboratory exercise is based on recognition of truth in testimony practices for forensic science training and is a start to blood identification exercises to establish if testimony matches the scientific evidence. For those interested in learning about forensic analyses, dissecting a court transcript to evaluate the veracity is an interesting introduction to the legal consequences to testimony and the need for accurate language. The value of linking laboratory work to courtroom testimony is the enhanced learning and ability to discuss the importance of being accurate in forensic diagnostic laboratory testing. There is a need to clearly communicate complex findings to the court and learn how to break down difficult science and technical terms into the clear communication of findings and this is a learned skill. By analyzing courtroom proceedings in transcript evaluations, the student can identify terms and phrases that are gaps in their understanding of the science that they are performing in the laboratory. In these two exercises, students are applying forensic science to determine if the concepts testified to were true and fairly represented. Microscopy can be used to screen possible blood stains and presumptive blood identification tests can yield positive results for nonvisible highly dilute bloodstains.

The MAR hearing was a legal proceeding designed to examine the quality and quantity of the evidence used for the post-conviction DNA testing in this case. The assessment by the judge was that although the evidence was stored inappropriately after the original trial, the value of performing the new DNA testing might shed some light on whether the substance was likely to be grease or blood. Why the confirmatory blood identification testing was not ordered for the coveralls by the court is still a perplexing mystery. Although some forensic laboratories have adopted the practice of using DNA to identify donor source after presumptive screening only, this practice lacks the specificity acquired through confirmatory tests to declare a substance as human blood based on the presence of human hemoglobin. At the time, there was also very little in the way of scientific research on what a bloodstain would look like under a microscope if it had been deposited by blood

spatter during the knifing incident as compared to transfer from bloody items during storage. One of the very valuable messages of this exercise is to show forensic science students that there are still areas of basic research needed to help bolster the use of forensic evidence and methods in the courtroom especially when using the science to answer questions of timing and manner of deposit.

One of the gaps in this case, *State of North Carolina v. George Goode Jr.*, was evident in that a peer-reviewed published scientific study was not available with photographs of bloodstains stored under a variety of conditions, ages and circumstances to aid the court in the determination for the timing of stain deposit. At the time, the scientific methods for determining the age of a bloodstain were not very well understood and this issue remains a challenge for forensic biologists today. There are two recent developments in the aging of bloodstains: qPCR for RNA degradation (10) and steady-state fluorescence spectroscopy (11). Quantitative PCR can be useful to measure RNA degradation by analysis of the degradation rate of multiple transcripts in a cell. Science has shown that the 5' phosphate group end degrades at a faster rate than the 3' hydroxyl group end of a DNA molecule. This type of assay has been shown to be useful for estimating timing of deposit within two to four weeks for bloodstains less than six months in age. For bloodstains between six and twelve months of age since deposit, the accuracy of this method is within four to six weeks from timing of deposit. This technique may not be useful on very old biological evidence but certainly could be useful if evidence is to be examined within the time frames specified. Fluorescence spectroscopy uses the decay in fluorescence of fluorophores such as tryptophan, NADH and flavins to measure the age of a bloodstain. Both methods are in the research and development phase and are not commonly used in United States forensic science laboratories yet. In the future, however, or for court-ordered cases, these techniques will hopefully be refined and fully validated for the use and interpretation of stains deposited during criminal acts.

Another issue that arose during this case was whether it was appropriate to combine blood-like stain cuttings together into a single tube for laboratory processing. By doing so, DNA mixtures were identified but could not be sourced back to a particular stain with any scientific certainty. In casework, combining samples from different stains could cloud the interpretation of blood stain patterns for donor sourcing. By creating false DNA mixtures by combining samples in the DNA testing process in the laboratory, information is lost. DNA test methods have become increasingly more sensitive with the polymerase chain reaction (PCR) method since the advent of forensic DNA analysis when restriction fragment length polymorphism (RFLP) was utilized. Therefore, there is often now no need to combine samples together to achieve

a readable DNA profile using current methods as compared to those methods used back in the 1990's.

Conceptually, the use of new DNA technology to identify if an old stain was biologically human blood is a misapplication of the science. It is further confounded if the evidence has not been stored in a pristine and controlled manner. DNA technology is so sensitive it can detect DNA molecules to the picogram levels and with modifications in PCR methods, can detect even as little as two picograms (approximately one third of a cell). The careful consideration of the context of the DNA to the case is vital and it is not always possible with older evidence and different handling practices in past eras to be sure that the DNA is from the crime event rather than from transfer during evidence collection procedures and the subsequent evidence transport and storage at the courthouse. More scientific research in this area would be helpful to further define and characterize the appearance of blood stain patterns from both direct deposit and transfer, for establishing the age of blood deposition and for developing better screening methods for post-conviction evidence that has been comingled or exposed to poor environmental conditions during storage.

The goal for this exercise was to link the courtroom transcript to the scientific techniques as a training exercise. By exploring testimony statements, students can learn about statements used to define the expert's experience, the techniques that were used, and why. Students could validate for themselves if the statements made at the MAR hearing appear to be true or false. Students can also consider the challenges of going back in time to different handling practices and standards used in forensic science when examining evidence for post-conviction information and cold cases. The conclusion for this case was a resentencing of the defendant from the death penalty to life imprisonment after post-conviction DNA testing.

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