CLS Investigation: Exploiting the Forensics Craze II

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ABBREVIATIONS: AAFS = American Academy of Forensic Science; CLS = clinical laboratory science; CODIS = combined DNA index system; DNA = deoxyribonucleic acid; FBI = Federal Bureau of Investigation; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphisms; STR = short tandem repeats; VNTR = variable numbers of tandem repeats.

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In part one of this article, an overview of forensics careers was presented, and some crime laboratory-type activities that might be within the capabilities of CLS programs were described. At our institution we've been successful with a program that includes a series of laboratory analyses that culminate in solving a "crime". This program is detailed below so that CLS educators can adapt it to particular needs and situations.

At our university, freshmen must participate in either a classroom or experiential orientation program. The university offers experiences such biking, sailing, and foreign travel that combine orientation with activity. Recently at summer's end we've done an experiential orientation program, Fun with Forensics, for 24 incoming freshmen. Over a few days, orientations sessions are interspersed with laboratory tests. We include special meals and socials and provide students with a Fun with Forensics T-shirt, creating walking advertisements for CLS.

To start, students are provided with a casebook that contains the facts of the case and a series of photographs of the crime scene and the body. For each laboratory test, there are a few paragraphs of background reading, a procedure, tables for data collection/calculations, and questions to guide students toward correct interpretations. An events schedule is included which specifies the various laboratory workstations. The students are divided into groups of six and each group has a customized schedule of laboratory rotation that allows faculty to work with one group or two, depending on the test. The case and schedule are constructed so that no one piece of information solves the whole case and no one group gets essential information earlier than the others. Virtually all results are needed at once to solve the crime, and the last results are simultaneously available to all students. Cases which include misleading or non-contributory laboratory results prevent any one result from being obviously important. This would certainly happen in real life, and it prevents anyone from spoiling the fun at the end.

Part of orientation at our institution consists of discussing academic success strategies, so we have built-in time to discuss choice of career, courses, majors, and minors. In this way we can ethically promote the CLS major by ensuring that we are not implying a CLS graduate is instantaneously employable in all forensics laboratories. We try to present the reality of career choices in forensics, CLS and other science majors, and twice we've had a Federal Bureau of Investigation (FBI) agent speak to the students on these issues.

A SAMPLE CLS FORENSICS CASE

The summer 2005 case involved a young woman found dead in apartment with a superficial head wound, defensive wounds on her extremities, a single traumatic puncture in

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the antecubital fossa of her right arm, and a kitchen knife in her left hand. The condition of the apartment suggested that a struggle took place, with blood spots near the body and the exit door. See Figure 1. In addition, a letter torn into several pieces was scattered over the apartment. The letter was collected as evidence, along with an empty syringe and red pills. See Figure 2. Information was provided from an interview with the victim's roommate regarding recent events in the victim's life and possible suspects. Three men were identified as Suspects #1, #2, and #3, and information from each was given with regard to their relationships with the victim and their movements prior to and after the estimated time of death.

Figure 1. One of the photographs of the murder victim provided to students in 2005 Fun with Forensics

Figure 2. Some of the evidence collected from the crime scene for the 2005 Fun with Forensics

A mock serum ethanol analysis was performed spectrophotometrically. The volatility of ethanol makes blood specimens more difficult to handle by the unskilled and many of the colorimetric reagents available utilize NADH UV endpoints. Because spectrophotometry might be a completely new concept to most students, different colorimetric procedures are so similar, and a visual endpoint is easier to understand, we used biuret for color and dilutions of bovine albumin for samples. This has the advantages of being inexpensive, non-volatile, less biohazardous, and visually clear at reaction's end. We've always included some level of "ethanol" in blood to provide an opportunity to discuss fermentation and postmortem ethanol synthesis. Final assessment of the victim's ethanol level at death is done by testing the vitreous humor from the eye, an anatomic site that decays less rapidly.[4] Since the volume of vitreous humor is small, screening for ethanol in blood is usually done first to conserve specimen. As a class demonstration, we inject into a gas chromatograph one uL to two uL of mock vitreous humor containing real ethanol and have students use this value as the true blood ethanol level at time of death. Colorimetric analysis could be done on vitreous humor, but using the small sample size and demonstrating another instrument is effective.

Blood typing is done on specimens from the victim, the suspects, and various parts of the crime scene. As the students are working in teams, it is easy to divide up the specimens and allow each student to type two to three specimens and pool the results. Students easily rule particular suspects in or out and/or identify a particular blood sample as coming from the victim or a suspect. Results are effortlessly made non-contributory if, for example, all the suspects are O+. If students believe it's contrived that all three suspects have the same type, faculty can engage in a discussion about blood type frequency and the implications to transfusion service clinical practice.

For trace evidence, we set up 14 microscopes with pre-prepared slides and found good fields to evaluate for each of the eight known samples and six unknown samples. Each microscope was labeled with its contents (Known hair: Suspect #1, Known carpet fibers: Suspect #2's car, Unknown hair: victim's body), and students circulated through the microscopes. Microscope workstations can be set up in advance, and since items like hair, carpet, soil, pollen, and powder are non-biohazardous, non-laboratory areas like conference rooms can be used and scopes left in place for additional review. Our microscopes are in a computer resource room so that students can review excellent trace evidence and fingerprint tutorials

developed by one of our faculty. With groups of students completing tutorials without supervision, faculty can closely monitor in-laboratory activities with other groups.

A fingerprint station can be set up in a non-laboratory area using black ink and paper or in the laboratory using the simple and inexpensive technique of iodine fuming.[2] In this case, we used the torn letter fragments as samples, and each student received a fragment for evaluation. Fresh iodine crystals were placed in the bottom of a stand-off jar, and paper fragments were inserted. The jar was placed on a mildly warm hotplate, and when fingerprints appeared, the paper was removed for comparison with the known fingerprints. That fingerprints are invisible so there is no way to know the quality of the prints prior to the laboratory presented a complication. We experimented with various hand lotions and soaps on our fingers to see which gave the best quality prints and ultimately found that a light coat of an antibacterial soap gave the sharpest and most reliable prints. The many paper fragments allowed enough students to get usable prints. An additional fun feature of our scenario was that the students could tape the letter back together to see what it said.

For shoe print analysis, we provided one pair of shoes known to belong to each suspect and the victim for comparison. We purchased inexpensive vinyl tiles, arranged them by the exit door in the crime scene photographs, and brought them to the laboratory for analysis. We mixed a staining solution for spray bottles in the following proportions: 0.2 g of amido black, ten mL of glacial acetic acid and 90 mL of methanol.[2]

Figure 3. A shoe print developed on vinyl tile using the amido black method for visualizing bloody shoe prints

The rinsing solution was comprised of 90 mL of methanol and ten mL glacial acetic acid.[2] We made the shoe prints by placing just enough water to cover the bottom of a tray and adding two mL to three mL of 22% bovine albumin. We carefully dipped the shoes into the solution and then made prints on individual tiles, allowing them to dry thoroughly. We covered the inside of a chemical fume hood with garbage bags, and students detected the prints on their tiles by first spraying them lightly inside the hood with the staining solution and then placing them in the rinse solution until the prints were clearly visible. Prints visualized this way are stable for weeks, so tiles can be retained for demonstration purposes. See Figure 3. Several other chemical formulas are available to detect bloody shoe prints,[2] but this protein stain was selected to avoid the use of blood. Actual bloody shoe prints could be made and developed using this or another reagent. An advantage to detecting bloody shoe prints over dirt shoe prints is that bloody prints would have likely occurred after the crime and could be more reliably indicative of a suspect's presence at the crime scene.

We did toxicology screening using a rapid screen (Triage®, a donation of expired cartridges) on the victim's urine, an extract of pills at the scene, and rinse solution from the empty syringe at the scene. Although rapid screening methods are expensive, they are easy to do in this format, and yellow-colored water spiked with drug standards/controls can be used for non-hazardous specimens. We have an excellent thin layer chromatography system (Toxi-Lab®) to use for confirmatory testing and identification of specific drugs, and once we identified specific drugs in the victim, we provided mock blood levels for each drug using microtiter well strips and a spectrophotometric strip reader . Students were shown that drug screening goes from rapid elimination or inclusion of broad categories of drugs (Triage®), to identification of specific drugs (Toxi-Lab®), and then to assessment of toxicity for individual drugs by blood levels.

We did deoxyribonucleic acid (DNA) analysis by restriction fragment length polymorphisms (RFLP) using a reasonably priced educational kit (Biorad®). Students digested and electrophoresed DNA samples from the crime scene, the victim, and all three suspects. The gels were left in a non-toxic stain overnight, and all students destained their gels at virtually the same time to visualize the bands and make a match. The RFLP method is a very accurate one but is less desirable for forensics because it requires a significant amount of DNA and time to complete. Polymerase chain reaction (PCR) analysis of highly polymorphic regions of

DNA with variable numbers of tandem repeats (VNTR) and short tandem repeats (STR) has been gaining favor for its speed and ability to detect small amounts of DNA.[2] However, this requires access to a thermocycler in addition to electrophoresis equipment. We have thermocyclers and have done VNTR analysis by PCR (Edvotek®) with our senior CLS students, but the mutagenic stain ethidium bromide is used and visualized under UV light due to the small amount of DNA present. Since the RFLP analysis has been very robust in the students' hands and the stain used is non-toxic, it is our personal preference to use for recruitment activities. In addition, the non-toxic stain requires no special light and the gels are more easily photographed. Educators should be prepared to discuss the national DNA database maintained by the FBI, CODIS (Combined DNA Index System),[2,5] to enrich this activity. CODIS has standardized 13 STRs for the forensics community to build an archive of DNA information to solve past, present, and future crimes and has many successful cases that can be discussed.[2]

In the summer of 2005 our victim was an epileptic prescribed phenobarbital to control her seizures. We identified phenobarbital in the pills but not in the victim. Vitreous humor testing for ethanol was negative, so blood ethanol was from post-mortem fermentation. The victim's roommate reported she had been taking "diet pills" and we found methamphetamine and its metabolite amphetamine in her urine. Since the metabolite was present, it is likely that she had taken the methamphetamine prior to her attack. Rinsing the syringe yielded cocaine, and cocaine was also found in her urine. Blood levels of cocaine, amphetamine, and methamphetamine indicated a fatal combination of stimulant drugs.

The roommate reported that the victim was right-handed, so the traumatic puncture wound on the right arm and the knife in the victim's left hand suggested that she had been forcibly injected with cocaine by someone else who tried to stage the crime scene with the knife placement.

The blood typing, trace evidence, fingerprint analysis, and shoe print analysis gradually allowed the students to construct a sequence of events around the three suspects that revealed a sordid tale of jealousy and anger. The crime scene DNA was taken from under the victim's fingernails, so presumably it was the murderer's. The DNA was the definitive piece of evidence to identify the correct suspect. Since this information was so crucial, it was the last test completed and all students interpreted the DNA bands at virtually the same time.

CONCLUSION

We change the case annually to prevent students from knowing the answer from the previous year. We do the same laboratory analyses, but we vary cases using accidental overdoses, unintentional lethal combinations of drugs/ethanol, and other crime scenes. For example, the victim's body has been placed in a car trunk and transported to the woods to be found later. This opens up all sorts possibilities with fibers from the trunks of the suspects' cars, soil samples from shoes, and pollen from plants. When a hair is pulled out rather than falling out naturally, the "bulb" end is present and can be used as a source for DNA rather than blood or tissue. In addition, it may be evidence of a struggle.[2] Structuring an interesting case requires only a good imagination and understanding of science.

As Fun with Forensics is scheduled over several days, it provides the luxury of interspersing non-laboratory activities with lengthy laboratory procedures to maintain student interest. As described in part one, however, we've been able to adapt the program in various ways to different audiences and time frames with great success.

While it might not be feasible to imitate our program exactly, it is highly likely that with some creative thinking and utilization of available resources most CLS educators could construct interesting recruitment activities that take advantage of the current interest in forensics. This would benefit not only educational programs but also prospective students by providing them with accurate and realistic information about career choices in science.

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