This is to certify that the

thesis entitled

FORENSIC SCIENCE IN THE HIGH SCHOOL CLASSROOM

presented by

Karen H. Pawloski

has been accepted towards fulfillment
of the requirements for

M.S. degree in Biological Science

Major professor

Date March 21, 1996

FORENSIC SCIENCE IN THE HIGH SCHOOL CLASSROOM

By

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THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Science Education

1996

ABSTRACT

FORENSIC SCIENCE IN THE HIGH SCHOOL CLASSROOM

By

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The high school science curriculum is in need of science courses which promote application of scientific concepts. In this thesis I have set about to design such a course based on forensic science and at the same time promote responsibility and higher level thinking among students. Included within the framework of the course is an emphasis on improving technical skills, the ability to work in groups and problem solving skills. I have integrated "real life" problems into the content. Assessment is through written work and oral presentations.

Dedicated to Dave, Sara and Jeffrey

ACKNOWLEDGEMENTS

The decision to develop this course, based on forensic science, was an easy one. Implementation was much more difficult. Several people kept me going, however, and without them I am not sure whether I would ever have finished.

The first is the staff and administration of my school district. They trusted me to develop this class without reservation. They gave me the funding necessary and encouraged me to continue. Second, Dr. Merle Heidemann always had a word of encouragement even when I was ready to quit. Dr. Jay Siegel, also, gently pushed and prodded me to continue. The "all points bulletin" on the internet was an eye opener for me. Dr. Marty Hetherington willingly helped me whenever I needed it including giving up lunch hours to meet with my students. Finally, I would like to thank my husband and my children. They have done everything within their power to encourage me to initiate and complete the document. THANKS TO ALL OF YOU.

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INTRODUCTION

PEDAGOGY

In 1932 a baby was kidnapped from a home in Hopewell, New Jersey. The child was that of Charles and Anne Lindbergh. Two months later the body of the child was found half buried in the woods. The public was shocked and the parents were devastated. Key evidence found at the home included a makeshift ladder which had been made of a variety of woods. Following two years of investigation and analysis a suspect was arrested and later convicted. The key investigator was a wood technologist. His most useful investigative tool was a microscope (Saferstein 169). This is science at its best.

The world is intrigued and fascinated by crimes and their investigations. From the Lindbergh case of 1932, John F. Kennedy's assassination in 1962, the Manson murders in 1969 to the Nicole Simpson and Ronald Goldman murders of 1994 the public has followed high profile cases with utmost interest. The average person has been exposed to science simply by listening to radio, watching television or reading the papers.
Discussions of bullet trajectories, blood types, stab wounds, writing samples and DNA have filled lunch rooms, coffee shops and workplaces. Human interest in science has been piqued by crimes and their solutions (or lack of). It is because of this interest that I have chosen to develop and evaluate a course on scientific application for my students (high school seniors) with its basis in forensic science.

The National Commission on Excellence in Education published "A Nation At Risk" in April 1983. This commission had been established by then Secretary of

Education, T.H. Bell in August, 1981 (United States: The National Commission on Excellence in Education 1). This resulted from "the widespread public perception that something is seriously amiss in our education system." (United States: The National Commission on Excellence in Education 1). The goal of the commission was to assess both college and high school teaching and learning and to compare American schools with schools in other countries. The document began with, "Our Nation is at risk." and within the first paragraph,

> We report to the American people that while we can take justifiable pride in what our schools and colleges have historically accomplished and contributed to the United States and the well being of its people, the educational foundations of our society are being eroded by a rising tide of mediocrity that threatens our very future as a Nation and a people. (United States: The National Commission on Excellence in Education 5).

In addition, a list of "Indicators of The Risk" was derived. These included inadequate performance on academic testing by American students when compared with other industrialized nations, 13% illiteracy of 17 year olds in the United States, declining SAT scores and...

> Many 17-year-olds do not possess the "higher order" intellectual skills we should expect of them. Nearly 40 percent cannot draw inferences from written material; only one-fifth can write a persuasive essay; and only one-third can solve a mathematics problem requiring several steps. (United States: The National Commission on Excellence in Education 9).

As expected, the Nation was in an uproar and changes in education began.

Promoting students beyond the knowledge and recall level of Bloom's Taxonomy became a priority. The overall goal of the educators, politicians and parents was to promote higher order thinking skills in all levels of education.

The ability to read and understand content area materials is one of the areas which needed and still needs to be improved. Currently, high school students in Michigan score poorly in this area on current assessment tests. It is the opinion of many experts that improving reading for understanding will promote reasoning skills, higher order thinking and problem solving.

> Using what they have read or heard as a catalyst for thinking about ideas, issues, and problems suggested in their reading requires that students engage in many skills listed in thinking taxonomies. They will need to evaluate evidence, draw conclusions, make inferences, develop a line of thought, etc. Students need to engage in such situations frequently, if we are going to help them develop the reasoning capabilities" (Beck 678).

Suggestions for improving reading and reasoning abilities include promoting background knowledge to the reader and focusing on problem solving (Beck 667-679). This needs to occur throughout the curriculum.

Improvement in the ability to write also has been established as extremely important. Journal writing has been shown to be a useful tool in promoting thoughtful writing and reflection and therefore promoting higher order thinking skills. Journal topics which begin with, "What if ...?", "This concept reminds me of ...?", or "In lab, I tried to figure out?..." can be used to stimulate thinking (Lozauskas and Barell 44). As stated in the article, Reflective Reading, by Dorothy Lozauskas and John Barell .

> Although it does take some time to read the journals, we gain insight into our students' general thought processes, their understanding or misconceptions about significant concepts and scientific processes, and even questions they were too embarrassed to bring up in class. The result is an enhancement of the teaching and learning processes (Lozauskas and Barell 44).

Besides promoting thinking, journal writing teaches the student to convey information in writing.

In addition to reading and writing as vehicles to promote higher order thinking skills, other strategies have been proposed. A very important tool is the ability to think and work as a team. Cooperative learning is a strategy which has been developed as a response to this.

Promoters of cooperative learning base the process on the idea that advanced learners can help less advanced learners and *vice versa*. By working in groups the students each have the ability to participate and, given proper structure, will participate. By formulating individual ideas and debating them as a team a higher level of problem solving can occur. This promotes higher order thinking for all of the students and encourages team work.

In science higher order thinking requires manipulation of variables and reasoning based on these manipulations. Yager and Penick (1987) suggested there is strong evidence that many science teachers continue to present science primarily by lecture and question and answer techniques (Yager and Penick 51). There is a definite need in science education to bring about a change in this by making a move towards more "hands on" manipulations in the classroom.

My goal when designing this unit was to take all of the above needs and suggestions and incorporate them into a science curriculum, specifically a semester long course. In addition I wanted to base the course on a topic which would promote learning science in an enjoyable, applicable and yet challenging manner. For these reasons I chose forensic science as the topic for this course.

Topics within the Forensic Science framework incorporate Biology, Chemistry and Physics. Therefore I chose to design this course for students who had completed most, if not all, of the above classes. The majority of students who take the course are in the top 25% of their class, but this is not required. I do not use a textbook. Lectures are rare but are used when necessary. The majority of the information learned by the students is through their own individual research, presentations by other students and laboratory exercises. Objective testing is not used. Subjective and oral testing are incorporated for evaluation. Students also demonstrate their knowledge through journal writing, research papers, analysis of unknowns, role play and class discussions. Participation is expected and evaluated.

This course taught in a rural school district located approximately 25 miles southwest of Lansing. I have taught it seven times, from Spring of 1993 to Fall of 1995. Students come from a variety of backgrounds. Some live and work on farms while others have parents who are professors and doctors that commute to the city to work. Class sizes range from 18 - 28 students. The administration is very supportive of this innovative and unique course.

INTRODUCTION

SCIENCE BACKGROUND

Several commonly used forensic techniques can be used to show how "scientific tools" can be used for solving crimes. These techniques include microscopy, chromatography, spectrophotometry and electrophoresis. Many types of physical evidence can be analyzed in the high school laboratory using these and other investigative techniques. I will briefly review each technique/equipment used in the unit. Further information can be found in Appendix A.

The microscope is considered the most important tool of the forensic scientist. There are many applications of the microscope within the forensic science framework for the high school laboratory. These include hair analysis, fiber analysis and document analysis. Many high school students do not have a strong background in the use of the microscope. Since much of forensic science is analyzed at the microscopic level this section of the course is very important. See Appendix A - Section II for further information on the microscope.

Chromatography, is a technique which can be used extensively in the high school laboratory, however, many students never get a chance to use it in a meaningful situation. I have used both paper chromatography and thin layer chromatography for analysis of inks, lipstick dyes and drugs. See Appendix A - Section III for more information on chromatography.

Spectrophotometry is another technique which is easily incorporated into the high school curriculum and which has many forensic applications. Blood salicylate simulations along with blood alcohol simulations both can be performed in the high school laboratory. See Appendix A - Section IV for more information on spectrophotometry.

Electrophoresis is an especially exciting technique which has great utility in forensic science. The students develop an appreciation of the complexity of DNA fingerprinting while completing the electrophoresis lab. In addition, these activities illustrate the integration of biology and chemistry. There are two electrophoresis labs in this unit. The first utilizes dyes as models of DNA and allows the student to gain pipetting experience before completing the second lab which uses DNA samples.

Many types of forensic evidence can be discussed and/or analyzed in the high school classroom. I usually divide the types of evidence into two categories, class and individual. See Appendix A - Section I for a detailed description of these classifications of forensic science.

Class evidence is that type which can be associated with a group but not an individual source. Examples include hair evidence, fiber evidence, blood and body fluids, DNA and toxicology (drug analysis). Individual evidence is that which can be associated with a specific source and includes fingerprints, shoeprint casts and questioned document analysis.

Hair is an excellent type of evidence to use in conjunction with the microscope. Not only does the student learn how to use the microscope and identify species of hair but also learns the importance of careful sketching and detailed notetaking. I am especially critical when grading this activity to ensure that the student understands the importance of keeping a detailed and thorough notebook.

Textile evidence evaluation is another activity which has many applications in the high school lab. The students continue to use the microscope yet begins to learn other techniques. These include burning fibers and evaluating residue, odor and the effect of vapors on litmus paper of the burned fibers. The fiber activities introduce the student to the idea that it may require several types of lab tests to identify a fiber or evaluate any specific piece of forensic evidence.

Blood and body fluids can and should be discussed in the forensic curriculum. There are many applications of blood and body fluids analysis including ABO typing, toxicology and DNA evaluation. An understanding of the ABO blood typing system is useful for anyone whether interested in forensics or not because of the many applications of the ABO blood type in all human lives. Lab activities using blood cannot be performed in the high school lab due to blood product restrictions but there are many simulations available. Body fluid simulations are also available for toxicology and drug evidence discussions. All forensic units should include a detailed discussion of DNA and DNA fingerprinting. If possible an electrophoresis lab of DNA should be included in this section. I have incorporated DNA discussions and labs into this course and because high school students, in general, have previously learned about DNA I have incorporate an advanced molecular discussion.

There are several categories of individual evidence which I have incorporated into this course. The students especially enjoy analyzing their own individual physical characteristics. This is the area of the course they find to be the most "fun". Examples include fingerprinting, making shoeprint casts and analyzing handwriting.

Fingerprinting analysis involves a minimal amount of preparation for the teacher and promotes several days of fun and learning for the students. This activity has several

parts. These include inking a set of 10 fingerprints, determining their FBI classification, dusting and lifting a print and making a 10 point print comparison. See Appendix B - Section IX for the fingerprinting lab. The activity does not require many materials and exposes the student to the individuality of the fingerprint.

That second type of individual evidence that is easily analyzed in the high school lab is shoeprint casts. Again, there is a minimal amount of required materials and this lab allows us to "escape" the classroom and go outside for a class period. The students also end up with a "make and take" project.

The third type of individual evidence is document and handwriting analysis. These labs require advanced preparation by the teacher (See Appendix B - Section VII for the document examination lab) but are enjoyable for the students. In addition, the labs provide the students with another example of the difference between class and individual evidence.

Other types of evidence which I discuss in the classroom but which I have not established labs for are Forensic Entomology, Forensic Anthropology, arson and glass and soil analysis. I do feel, however, that all of the above have high school lab applications and would provide future direction for continued improvement of this course.

Besides techniques and evidence, a thorough discussion of the crime scene should be a part of all forensic lab units. Students need to have an understanding of all that is involved in the collection and preparation of evidence to fully understand its application in crime solving.

STUDENT EXPECTATIONS

TECHNIQUES

Microscope

The students are expected to:

1. be able to properly use the microscope.
2. determine the overall magnification of each objective.
3. make detailed sketches of all microscopic observations.
4. discuss the difference between compound and stereomicroscopes.

Spectrophotometer

The student is expected to:

1. properly use and explain the use of the spectrophotometer.
2. read and record the absorbance of a sample.
3. determine the concentrations of serial dilutions.
4. use absorbance data to create a graph of absorbance vs. concentration.
5. use their graph to determine the concentration of an unknown.
6. explain how the spectrophotometer can be used to determine salicylic acid concentrations and ethyl alcohol concentrations.

Chromatography

The students are expected to:

1. explain the process of chromatography (stationary vs. mobile).
2. use paper chromatography to analyze water soluble pen inks.
3. use thin layer chromatography to analyze ball point ink dyes and lipstick dyes.
4. perform and explain Rf calculations and their use.

Electrophoresis

The students are expected to:

1. explain the electrophoresis apparatus and procedure.
2. perform electrophoresis on a variety of dyes and interpret gels.
3. perform electrophoresis on a DNA sample and interpret gels.

STUDENT EXPECTATIONS

ANALYSIS OF FORENSIC EVIDENCE WITH LAB ACTIVITIES

Hair

The students are expected to:

1. analyze the morphological characteristic of hair from various animal sources.
2. determine the animal source of an unknown hair.

Textile Fibers

The students are expected to:

1. analyze the characteristics of natural fibers.
2. analyze the characteristics of synthetic fibers.
3. compare and contrast natural and synthetic fibers.
4. use a variety of techniques to determine an unknown fiber type.

Blood and Body Fluids

The students are expected to:

1. Perform a simulated ouchterlony procedure to determine whether a stain is or is not human blood.
2. Perform a simulated urine analysis to determine the types of drugs present in a sample.
3. Use electrophoresis to perform DNA fingerprinting.

Lipstick Dyes

The students are expected to:

1. explain the chemical make-up of lipstick.
2. use chromatography to determine if one of several lipsticks matches that of a suspect.

Ink Dyes

The students are expected to:

1. Analyze water based dyes using paper chromatography to determine the identity of the pen used to write a threatening note.
2. Analyze ball point ink dyes using thin layer chromatography to determine the identity of pen used to write a threatening note.

Fingerprints

The students are expected to:

1. ink a set of 10 fingerprints.
2. classify each of the 10 prints in #1 and perform an FBI primary classification.
3. dust and lift a print.
4. compare a matching inked and lifted print to 10 points.

Shoeprints

The students are expected to:

1. make a plaster cast of their own shoeprint.
2. explain why shoeprints are individual evidence.

Questioned documents

The students are expected to:

1. match the writing on a suspect note to a known exemplar.
2. perform a tracing and a forgery on a friends signature.

STUDENT EXPECTATIONS

ANALYSIS OF FORENSIC EVIDENCE WITHOUT LAB ACTIVITIES

Forensic entomology

The students are expected to:

1. explain the use of entomology in solving crimes.
2. give an example of entomology use in crime solving.

Forensic anthropology

The students are expected to:

1. explain the use of anthropology (bone structure) in solving crimes.
2. give an example of anthropology use in crime solving.

Arson

The students are expect to:

1. explain the chemistry of fire.
2. discuss various types of arson accelerants.
3. explain how accelerant analysis can be used in solving a crime.
4. explain how arson investigators determine the cause of a fire.

Glass and soil evidence

The students are expected to:

1. explain why glass and soil evidence are both class evidence.
2. explain why William Kennedy Smith was acquitted of rape charges.

STUDENT EXPECTATIONS

OTHER ACTIVITIES RELATED TO FORENSIC SCIENCE

The Crime Scene

The students are expected to:

1. develop a thorough understanding of all parts of the crime scene including preserve and protect, and maintaining a chain of custody.
2. participate in a crime scene role play activity. (See Appendix A - Section I for more details).

Case Readings

The students are expected to:

1. participate in the reading and analysis of the Lindberg Kidnapping case.
2. participate in the reading and analysis of the Wayne Williams case.

IMPLEMENTATION

The decision to teach this unit as a semester course in our science curriculum was a fairly easy one to make given the enthusiasm and support on the part of the many people involved. First and foremost I questioned my Chemistry I students about their opinions for a new upper level science course. The basic concern of the students was that it not be "another lecture course", that they could learn science by application and that they could have fun. Second, I talked to the other staff members in the department and they were 100% supportive of an applied type of class and finally, the faculty at Michigan State University were willing to help in any way.

The labs and activities which I have incorporated are a combination of activities from a variety of sources. Some remain as they were written in their respective lab manuals. Many have been adapted from a variety of lab manuals for specific use in my classroom and a few I have designed myself. Specifically, the activities which I designed include the suspect note investigation of the Paper Chromatography lab, Crime Scene A and Crime Scene B of the Microscope Lab, the crime scene investigation in the Examination of Hair by Light Microscopy lab, the Carpet Sample Lab, the crime scene in the Lipstick Chromatography lab, the Determination of the Peak Absorbance of a Substance Using Visible Spectrophotometry lab, and the Crime Scene Role Play activity. Activities which I did not design but revised for use in my classroom include the Practice in the Use of the Microscope lab, Examination of Hair by Light Microscopy, Analysis of

Fibers, Determination of Lipstick Dyes Using TLC, Analysis of Inks by Thin Layer Chromatography, Identification of Salicylates in Blood by Spectroscopy, The Alcohol Breathalyzer Test: A Laboratory Simulation, and the DNA Electrophoresis lab. I will be discussing each of them in the order that I use them in my classroom.

In addition, I have incorporated many methods in my unit including lectures, projects, audio-visual aides, current event topics, case readings and role play activities. Basic class requirements per nine week (two nine week marking periods per semester) marking period are listed below:

Attendance and participation	200 points
Notebook	200 points
Project	200 points
Lab exercises and unknown solutions	200 points
Crime scene role play (second marking period only)	100 points
Total	900 points

First marking period has approximately 800 points.

Each students is required to keep notebooks of all class activities. Included in this notebook are lecture notes, handouts, case reading analysis, current event discussions and lab activities. The notebook must be in chronological order and detailed. I tell the students to pretend that they are "real" forensic scientists and could be called to the witness stand at any time. Their notebook may be their only recollection of the evidence involved and therefore should be as complete as possible.

Attendance and participation is another important aspect of the course. I feel that in order to successfully understand and complete the many classroom activities the student

must be in class and participating. Copying of other students lab sheets is not acceptable.

The student must complete a project each marking period. For the first nine weeks this is a group project. The second nine weeks' project is individual. The group project usually involves writing a research paper, developing a visual aide and making a presentation to the class. Examples of research topics have included evidence investigation (fibers, DNA, blood typing) and case readings and analysis (Manson murders and the John Wayne Gacy trial). No two groups can use the same topic and therefore each group learns from the presentations of other groups. In general, the students do a thorough job on their projects. I have seen some very unique and creative presentations.

Individual projects are usually designed by the students themselves. Current event topics have been a favorite choice. In this instance students must follow current event situations involving crime and criminal evidence and write an overview of the situations. Other choices have been critiques of suspense or thriller novels and writing their own crime story.

The fourth area students are graded on is their laboratory write-ups and unknown investigations and evaluations. They must justify their conclusion regarding their unknown with their laboratory procedures.

Lastly, they are graded on their participation in the crime scene role play activity during the second marking period. We begin this activity after the students have completed a majority of the labs.

For the final exam I usually put the students on the witness stand with their notebooks. I randomly ask them questions regarding the semester work which they should be able to answer correctly if they have done a good job on their notebooks. They are graded on their responses.

COURSE OUTLINE

I. Lecture topics

- A. The Crime Scene
  - 1. outline
  - 2. guest speaker
    - a. crime van
    - b. forensic entomology
    - c. forensic anthropology
    - d. interrogation
  - 3. crime scene role play
- B. Hair morphology and analysis
- C. Fiber morphology and analysis
- D. Document examination
- E. Fingerprint examination
- F. Toxicology
- G. Blood and Body Fluids
- H. DNA

II. Group Discussions/ Work

A. Current Events

1. Topics brought up daily and evidence discussed

2. Individual project - second nine weeks

B. Review of past cases

1. Lindbergh kidnapping case

2. Wayne Williams murder trial

3. John Kennedy assassination

III. Laboratory Exercises

A. Paper chromatography

1. Water soluble ink analysis

2. Toxicology

B. Hair Analysis

C. Fiber Analysis

D. Thin Layer Chromatography

1. Lipstick Analysis

2. Ink Analysis

E. Document Examination

F. Fingerprint Evaluation

G. Ouchterlony - blood analysis

H. Spectrophotometry

1. Graphing exercise

2. Testing for salicylates in blood

3. Breathalyzer simulation

I. Electrophoresis

1. Dyes

2. DNA

J. Footprints

IV. Audio-Visual

A. 118 Green Street - NOVA program

B. Sherlock Holmes videos

V. Field trip

A. Michigan State University forensic laboratories

B. Michigan State University glass blowing laboratories

C. Michigan State University Museum

VI. Guest speakers

A. Detective

B. Arson investigator

C. Michigan State University forensic scientist

D. DARE officer

VII. Evaluations

A. Lab unknowns

B. Detailed Journal

C. Projects

1. Group - first nine weeks

2. Individual - second nine weeks

D. Attendance and daily participation

E. Crime scene role play

F. Final exam

1. Simulated jury trial

2. Individual expert questions

LABORATORY EXERCISES

PAPER CHROMATOGRAPHY

Paper chromatography is an excellent lab to use at the very beginning of the semester. I have adapted it for use in my classroom from a high school chemistry laboratory manual. See Appendix B - Section I for a detailed write-up. It serves several functions. First, because the students are required to use it to solve a crime, it piques their interest in regards to the rest of the semester. Second, chromatography has a strong application to biochemistry and forensic science and it requires the students to begin thinking at the molecular level. Third, the students are always amazed at the results.

This lab is based on the fact that black, water soluble inks are made up of different combinations of colored ink dyes. Different companies use different combinations and therefore by analyzing a series of "known" ink pens with chromatographic methods a student can identify the pen that an "unknown" ink mark was made by.

The students not only have to carefully follow directions in the lab but they also have to figure out a way to analyze the unknown ink. This ink is written on a piece of notebook paper. The students have to determine how to transfer that ink to filter paper in order to chromatograph it. Finally, one of the "known" pens is a permanent marker which is insoluble in water. This requires them to evaluate why the one dot didn't move but the others did.

Overall, the lab is outstanding. It relates to real "forensic" work. It includes critical thinking and evaluation. I usually begin discussing the techniques of chromatography after this lab is completed and refer back to it. The lab can be completed in two 50 minute class periods.

PRACTICE IN THE USE OF THE MICROSCOPE

The microscope is used extensively in forensic science and many people believe it to be the most valuable tool. The students, therefore, need to have a thorough understanding of it's make-up and use. This lab requires the student to learn and use both a compound microscope and a stereo microscope. I have adapted this lab for my classroom from a forensic science lab manual. See Appendix B - Section II for the lab write-up.

The students are required to look at and sketch a letter from the newspaper using both types of microscopes. They then make a comparison between the two images. Upon completion of this they are given two crimes to solve. One requires the analysis of paper matches found at a crime scene. The other involves a college student who is accused of cheating on a test. The students analyze the ink samples on the test. Each student is given their own unknown to solve. They must have a thorough understanding of the lab procedures in order to identify their unknown. Students are evaluated on their final unknown determination and on their lab write up. This lab is very effective because of it's ability to introduce the students to the microscope and incorporate the critical thinking aspect of crime solving. The lab can be completed in two 50 minute lab periods.

EXAMINATION OF HAIR BY LIGHT MICROSCOPY

This lab is a combination of several hair identification labs. I have incorporated those procedures which seem to work the best into one lab. The students are required to analyze and evaluate four types of animal hair; human, dog, cat and horse. This analysis includes both gross and microscopic examination of the hair. In addition, the students are given trace hair evidence which was taken from a crime scene and are asked to determine its origin.

The students are always amazed at their ability to determine the species from which their hair sample came. They also enjoy seeing all of the "junk" (hairspray, gel, etc.) that is clinging to their own hair. I especially like this lab because it requires the student to use the microscope, note details, critically observe what they are seeing and prepare detailed sketches. The average high school student does not get a lot of experience doing this. This lab can be completed in four 50 minute lab periods. See Appendix B - Section III for a detailed write-up.

ANALYSIS OF FIBERS

As with the hair analysis lab, the fiber lab is a combination of parts of several labs. It continues to give the student experience using the microscope and utilizes other technical skills as well. Besides microscopic evaluation, the students analyze the effect heating the fibers has on litmus paper, the residue left from burning the fibers and the solubility of the fibers in different solvents. The students are required to evaluate the difference between natural and man-made fibers based on all of the above characteristizations.

This lab requires a great deal of reinforcement and/or encouragement by the teacher. It takes one to two weeks to complete and sometimes can get monotonous for the student. The students do learn a great deal from this lab, however. In addition, it helps the student to see an connection between chemistry and biology. Though this is not a favorite lab for the students they do comment on the amount of knowledge they gain from it. See Appendix B - Section IV for the lab write-up.

CARPET SAMPLE UNKNOWN

At the conclusion of the hair and fiber labs each student is given a carpet sample to investigate. Imbedded in the carpet are several hair or fiber samples of one type. The student must find the samples and analyze them according to the procedures in the previous two labs. They must determine the type of hair or fiber they have and give a detailed explanation of their analysis.

This activity incorporates both the hair and fiber labs. The students each have their own unknown so they must rely on their own knowledge to solve the problem. This activity can be completed in one 50 minute lab period. See Appendix B - V for the lab write-up.

DETERMINATION OF LIPSTICK DYES USING THIN LAYER CHROMATOGRAPHY

This lab is an introduction to a more technical type of chromatography used by the forensic scientist. The day of this lab the students enter the classroom and find a message scrawled across the window which was written using red lipstick. I tell them that I have confiscated the lipsticks from three teachers who I suspect wrote the note. They are required to analyze the known lipsticks and the lipstick from the window using thin layer chromatography.

This lab is an excellent teaching tool for several reasons. First, it allows for a discussion of lipstick components. Many students find it disgusting to learn that they are putting metals mixed with animal lipids onto their lips when they apply lipstick. Second, because the solvent used has an especially bad odor we perform the chromatography in the fume hood. The students always enjoy "working in the hood". Finally, with the determination of Rf values for the resulting bands a true relationship between mathematics and science is established. This lab can be completed in two 50 minute lab periods. See Appendix B - Section VI for the lab write-up.

ANALYSIS OF QUESTIONED DOCUMENTS

Throughout the first marking period I periodically collect writing samples from the students. The samples are dictated to them. Sometimes I dictate fast, sometimes slow and sometimes I ask them to try to disguise their handwriting. I then select one of the samples from each student to be a questioned document. I also select four other samples per questioned document, one of which was written by the same writer as the questioned document and three which have similar writing but are not exact. Each student gets one of these sets and no student gets their own writing for their questioned document. The student must determine which of the four samples was written by the same person as their questioned document. They must match the two documents with ten points based on the characteristics which were talked about in lecture.

The students usually enjoy this activity. They are always surprised that even though they tried to disguise their writing some characteristics still come through. The set up for the lab requires a lot of time but it is an effective way of showing the individual nature of handwriting. This lab requires three 50 minute lab periods to complete.
See Appendix B - Section VII for the lab write-up.

ANALYSIS OF INKS BY THIN LAYER CHROMATOGRAPHY

This lab is used in conjunction with the Questioned Document lab. Analyzing the inks are an important part of questioned document evaluation. The lab is very similar to the water soluble ink lab but utilizes organic solvents and the techniques of thin layer chromatography.

As with water soluble inks, ball point inks have their own unique components. The band patterns which result from this procedure are more complex than the lipstick analysis chromatography. The lab is solved by a comparison of Rf values. See Appendix B.

The students again get the experience of working in the fume hood and of incorporating mathematical concepts into science. The lab is easy to follow and complete and I feel it is effective in portraying the unique components of different pen types. This lab requires two 50 minute lab periods to complete. See Appendix B - Section VII for the lab write-up.

VISUALIZATION AND EXAMINATION OF FINGERPRINTS

Fingerprints are everywhere. Students have seen and read about fingerprint evidence over and over. Rarely, however, do they have a biological and physical understanding of fingerprints.

This lab requires them to do several activities with their own and each others fingerprints. They first must ink and roll a partners set of ten prints on an FBI card. They

then must classify all of their own prints as to print type. From this determination they can then determine their FBI primary classification. In addition, they are required to dust and lift a print. Finally, they must identify their own thumb print from a sheet of approximately 25 (their classmates) thumb prints. They must match this print to an inked print with 12 points.

Many students say this is their favorite lab. All of the evidence is theirs and they are amazed at how unique they are. They learn a lot about comparison techniques and the detail involved in some forms of science. This lab requires three to four 50 minute lab periods. See Appendix B - Section IX for the lab write-up.

SHOEPRINTS

The shoeprint lab is another way to demonstrate individual evidence. The lab involves taking the students outside and making plaster casts of their shoeprints. The casts are then brought inside and I demonstrate making ink impressions with one of the prints. The students determine that even if they have the identical type of shoe as another student their shoeprint is different.

I do not have each student ink their own cast. This simply gets too messy. In addition many of the students want to take their print home. This would be impossible if the cast was inked. This lab can be completed in one to two 50 minute lab periods. See Appendix B - Section X for the lab write-up.

PAPER CHROMATOGRAPHY - TOXICOLOGY

Drug analysis in a high school classroom is impossible so I use this simulation instead. This lab involves paper chromatography and food coloring. It is very similar to the other paper chromatography lab we do except that we use paper chromatography paper instead of filter paper. The food coloring portrays urine samples with drugs in them. The students analyze the different colors which represent various drugs and base their conclusion on the results. This lab can be performed in one hour. A possible improvement to this lab would be to use over-the-counter drug preparations instead of food coloring. See Appendix B - Section XI for the lab write-up.

OUCHTERLONY DIFFUSION

Blood products are often found at the crime scene but the analysis of blood is not allowed in the high school laboratory. This technique involves the chemical simulation of human blood and human blood antiserum. The lab includes the making of ouchterlony media, preparing and loading the wells and observing precipitin reactions. It fits into a blood and body fluids discussion. After this activity I usually discuss A-B-O blood typing procedures. Overall, the lab is a good experience for the students. The results sometimes are vague and difficult to interpret, however. One possible solution would be to use horse blood and buy anti-horse serum. The test could then be run using real blood. This lab can be completed in two 50 minute lab periods. See Appendix B - Section XII for the lab write-up.

DETERMINATION OF PEAK ABSORBANCE USING THE SPEC 20

This lab is performed before any other labs are done with the Spec 20. The students are required to prepare a series of dilutions using methylene blue stain. They then determine their % transmittance and absorbance of each dilution at varying wavelengths. After plotting this data they are able to see that there is a common absorbance peak between each concentration. This is the maximum absorbance.

The major drawback of this lab is the time is takes to complete. At least three days should be allowed for completion. The tubes must be covered with parafilm and wrapped in foil if left overnight. The results are very good however and the students begin to understand how wavelength values are determined. See Appendix B - Section XIII for the lab write-up.

IDENTIFICATION OF SALICYLATES IN THE BLOOD BY SPECTROSCOPY

The use of a spectrophotometer at the high school level is unique for many students. Several of my former students have returned from college and told me that they were the only ones in their college chemistry class who knew how to use this instrument. The drawback of Spec 20 labs for me is the school does not own them. The only way for me to do the labs is to rent and transport the machines.

Students identify blood salicylate levels in a simulated blood sample. The lab requires the students to make a series of ferric nitrate/salicylic acid dilutions, determine the

concentrations of each dilution, test them for percent transmittance, prepare a calibration curve and determine the concentration of an unknown salicylic acid sample. After successful completion of this procedure they are given a crime scene situation and must use their information from the first section to complete the second.

The students learn how to correctly plot data and then use this data in this lab exercise. The lab works well. It takes approximately two 50 minute lab periods to complete. See Appendix B - Section XIV for the lab write-up.

THE ALCOHOL BREATHALYZER TEST: A LABORATORY SIMULATION

The students find this exercise, which also uses the Spec 20, to be especially interesting because of the widespread use of the breathalyzer test. The students make a series of ethyl alcohol/potassium dichromate dilutions of known concentration, plot their data and then determine the concentration of an unknown ethyl alcohol sample.

The procedure is very simple to follow and the results are consistent. Potassium dichromate is a suspected carcinogen and the students need to know to use caution when working with the chemicals. The concentration of the chemicals they work with are low (compared to techniques used in their previous chemistry labs) and there is a greater chance of experimental error with this lab due to this. This lab takes approximately two 50 minute lab periods to complete. See Appendix B - Section XV for lab write-up.

AGAROSE GEL ELECTROPHORESIS USING DYES

This lab is a great introductory lab to electrophoresis because of the low cost of the dyes used. It gives the students practice using micropipettes which is helpful when we do the DNA electrophoresis lab.

The students are required to make their own gels, fill the wells and electrophorese the dyes. In addition they are given a mixture of dyes as an unknown and they are required to separate these using electrophoresis. From the electrophoresis results they can determine the components of the unknown dye mixture.

This is an excellent laboratory exercise. The results are always consistent. The students develop an appreciation for the fact that molecules can be either positively or negatively and that they can separate mixtures based on this property. The lab usually takes two 50 minute lab periods to complete. See Appendix B - Section XVI for the lab write-up.

DNA ELECTROPHORESIS

With the high profile use of DNA fingerprinting in crime solving today an understanding of the process is useful for all of us. At this point in the class the students have developed an understanding of correct experimental procedures and are ready to work with a complicated and expensive molecule like DNA. Again, they prepare their

own gels, load the wells with the sample and electrophorese them. In addition, they must stain and de-stain the gels in order to visualize the DNA fragments.

The lab is very simple to follow, can be completed in two lab periods and gives the student a basic understanding of DNA fingerprinting. See Appendix B - Section XVII for a detailed lab write-up.

OTHER ACTIVITIES

SPEAKERS

I usually have three to four speakers come into my classroom each semester. This allows the student to see individuals who are currently working in forensic related fields. Types of careers presented include detectives, DARE officers, arson investigators and forensic scientists.

The Livingston County Sheriffs Department has several detectives who are willing to speak to my classes. The detectives usually bring the crime van to the school so that the students have an opportunity to observe pieces of equipment and other items which are taken to crimes scenes. In addition the detectives will talk about criminal cases, evidence and areas of investigation. I usually ask them to talk about the areas of evidence which we do not cover in class like forensic anthropology, forensic entomology and autopsies. A discussion of what happens in an interrogation room is also a topic of interest for the students.

The DARE (Drug Awareness Reinforcement Education) officers also are commonly seen in our schools and are willing to talk to the students. They will usually discuss the drugs of choice in the area for students their age and the dangers of these drugs. They will also discuss what the area police departments are doing to combat the drugs and some of the current drug laws. Many times they will drive a very expensive automobile to the school and explain to the students that it was confiscated in a drug raid and now belongs to the department.

Arson investigation is another area of interest for my students and there is an investigator in Livingston County who will speak to the students. This individual does an excellent job of teaching the students that fire and arson are chemistry. In addition he discusses how fire investigators can determine the origin of a fire by burn patterns on the floors, walls and ceilings. He also shows them how light bulbs can be especially helpful in determining fire origins because of the many components which burn at very different temperatures. Students find this discussion very interesting and informative.

Dr. Jay Siegel, professor of forensics at Michigan State University is willing to speak to the students, also. The students rarely have had any exposure to college professors and therefore find this lecture to be especially interesting. He is able to portray to the students the fact that professors are real people. Dr. Siegel speaks to the students as adults and this is very much appreciated by them. He gives many examples of forensic science use in crime scene analysis. The students gain a lot of insight into forensic science from this presentation.

FIELD TRIPS

I always try to take the students on a field trip to Michigan State University. The faculty are very willing to give presentations and help with this in any way. Areas which I have found to be especially interesting are tours of the chemistry and forensic laboratories, tours of the glassblowing laboratories, greenhouse tours and museum tours.

Dr. Siegel will usually give the forensic science and chemistry lab tours. He shows them the many advanced pieces of equipment used in the forensic lab. They probably would not have this opportunity if not for the willingness of Dr. Siegel to help. In addition, the students get to see what a college laboratory and lecture hall look like. For many of them this is the first time they have ever been in a college classroom.

The glassblowing laboratory is usually the second stop on our visit. The students get to participate in the art of glassblowing. They are very enthusiastic about this tour and talk about this for weeks.

The day usually ends with a visit to either the greenhouses or the museum. Both tours are outstanding. The diversity of plants which can be found in the greenhouse is always a point of discussion after the tour. Several students have commented on their intent to go into botany after this tour. The museum is also a very interesting tour. We usually get the opportunity to visit the "behind the scene" libraries where many of the research animals are kept. The students enjoy seeing drawer after drawer of preserved animals. Occasionally the students also get to observe the Dermestids lab. This has a great application to forensics anthropology and again is a great area of discussion after the field trip.

AUDIO-VISUAL AIDES

Many of the crime videos available are simply too graphic to be used in a high school classroom. However, two that I have found to be useful are 118 Green Street by NOVA and Sherlock Holmes videos.

The video 118 Green Street (NOVA) is a great representation of the microscopic world. The students develop a respect for the microorganisms around us from this movie. I usually will show this during the hair and fiber laboratories.

Sherlock Holmes videos can be shown anytime. Some of them tend to be more explicit than others and should be screened before showing. The videos, however, do have a great deal of evidence. Most of this evidence can be seen with the naked eye but only the creative expert thinks to use it. The students begin to develop the idea that much of science is actually a creative thought process and that higher level thinking can help them evaluate many situations.

CASE READINGS

I usually have the students read at least three case readings. The choices I use are the Lindbergh kidnapping case, the Wayne Williams murder case, and the John F. Kennedy assassination. The students have usually only heard of the latter case but do develop an interest in the former cases once they read them.

I like to use the Lindbergh case reading because of the extensive use of the microscope in examining the evidence. After reading this case the student learns how valuable a tool the microscope is. They also find it very interesting that a person can be such an expert in a subject that the majority of the population knows very little about. Besides talking about the crime I use this case to talk about how important it is to become an expert in the career they choose to undertake.

The Wayne Williams case reading is very long and sometimes difficult to understand. However, it does show the student how class evidence can be used in conjunction with other evidence to convict an individual.

The John F. Kennedy case reading allows the student to evaluate what they feel really happened. With so many conflicting stories surfacing about this assassination the students get the opportunity to look at the evidence and develop their own conclusion.

ROLE PLAY

CRIME SCENE

One of the favorite activities of the class is the crime scene role play. The class is divided into two parts, group A and group B. Group A designs a crime for Group B to solve and *vice versa*. The crime must be well thought out with at least three pieces of evidence involved. Every individual in the group must have a part in the crime even if it is a minor one. All members must have a thorough understanding of their own part, their own crime and the other members' parts.

After the initial design phase one of the groups is chosen to set up their crime scene. While they are doing this the other group must prepare to solve the crime. Each member of the solving group must have a role. Roles include first officer at the scene, chief investigator, detective, note taker, sketch artist, photographer, news paper reporter, interrogator and forensic scientist. They must know the requirements of their part and be able to perform the part well.

During implementation I watch each member of the role play carefully. The students are graded on their set-up and their performance. For example, if Detective A forgets to bring his crime scene kit (tweezers, gloves, evidence bags etc.) to the crime scene, he will have points taken off his total score. Also, if suspect C forgets that she was actually at the neighbors house during the crime not in the neighborhood bar she will have points taken off her total score. The students are expected to have a thorough

understanding of their part in the role play and be prepared to carry it out correctly. After this case is solved the two groups reverse roles and the second crime is set-up and solved.

This activity is outstanding. The students really have to become critical thinkers to solve the crime. They get frustrated with each other and usually have to go back to square one and start over. Team work becomes very important to them and they get disgusted when one member does not do their share. I feel this activity is as beneficial to them as the labs are.

EVALUATION

The material presented in this unit is different from the material presented in any other class we offer at our school as it includes many activities that are subjectively evaluated. Therefore, pre and post testing is not very useful. I have given pretests before and the students usually indicate that they do not have any or very little knowledge of the topics we discuss. Student interviews and discussions, however, seem to be an effective form of evaluation.

There are several different aspects of the class that the students have indicated as being "what they liked most". These include being able to work at their own pace, being able to socialize with other students while doing school work, less amount or time spent in lecture, doing lots of labs, learning material that they can apply to real life situations and specific areas that interested them.

For many of the lab activities I give the lecture presentations, a pre-laboratory discussion, and a time line for completion. The students then can work at their own pace. Students know that if they waste a lab day that they either have to work at twice the pace the next day or come in after school to make up the lost time. If they get done before the due date then they can use the time to work on their nine weeks project. This is the only time that they are given in class to work on the project. The students appreciate this sense of trust. Some of them learn that they need a more structured environment and ask to be given more deadlines, but the majority of them get the work done when it needs to be done.

This type of environment promotes a lot of socialization among the students. They really become a cohesive unit. The class is only offered to seniors and therefore this may be one of their last chances they get to spend time with other students. I, also, enjoy being able to interact with students in a less formal atmosphere.

The minimal amount of lecture is also appreciated by the students. My goal with lecture is to introduce as much material as I can while using a minimal amount of class time. In many instances I give them written material instead of lecture. Those who have questions can meet with me individually. The majority of the students are high achievers and they make sure they get the information they need to get the job done.

Students appreciate the fact that they are being taught a science that they can see has a direct application to their lives or at least lives they see in the media. Many of the comments I hear deal with the fact that they could understand the material because they heard about it everyday in the news. They enjoyed knowing just a little bit more than the average citizen.

Students also enjoy doing labs. They do, however, have their definite likes and dislikes. Fingerprinting tends to be a favorite. They magnify their print and locate many of the points of comparison. In addition, viewing the fingerprint under the stereo microscope brings about a lot of enthusiasm. "Wow, you can actually see the oils on there!" The fiber lab tends to be one of the least favorite. Many factors contribute to this. The lab takes about two weeks to complete and therefore gets monotonous. The fibers have a lot of unique smells which tend to turn the stomachs of a few and the difficulty in manipulating the fibers gets frustrating.

Some of the comments from the Fall 1995 semester interviews include:

"Chemistry II allows students to see how science works in real life making the educational experience much more exciting."

"I feel I have learned more in this class than any other."

"I like doing the labs because as we do it we are learning and don't even know it."

"It was a great semester. It is fun and you learn."

"We learned alot of important things and we also had fun."

"I liked it and I did actually learn something."

"I enjoyed the openness between the teacher and students."

"I learned plenty and I'm sure other people will too."

"Chemistry II encourages group work, open thought and discussion and has much to offer in the ways of forensic science."

In addition to class discussion I asked the students to evaluate their knowledge on a variety of topics. They were instructed to address this both before taking and after completing the class. They graded their knowledge on a scale of 1 - 5 with 1 being they knew nothing about the subject and 5 being they knew a lot about the subject. Twenty three students completed the questionnaire. The average results are as follows.

Table 1
Percent change of a variety of studied topics.

BEFORE	TOPIC	AFTER	% CHANGE
2.78	light microscopy	4.48	61%
1.43	stereo microscopy	3.48	143%
1.96	chromatography (general)	4.34	166%
1.78	paper chromatography	4.34	144%
1.61	thin layer chromatography	4.09	156%
1.48	water based ink dyes	4.09	176%
1.48	ball point ink dyes	4.26	188%
1.48	lipstick dyes	4.26	188%
2.13	crime scene procedures	4.71	120%
2.35	arson	4.01	70%
2.57	DNA evidence	4.04	61%
2.57	blood and body fluids	4.31	67%
1.57	hair evidence	4.39	180%
1.43	fiber evidence	4.22	194%
2.52	drug categorization	4.09	62%
2.74	drug laws	4.13	51%
2.31	fingerprinting	4.78	108%
1.31	forensic entomology	3.31	154%
1.31	forensic anthropology	3.04	134%
1.51	document evaluation	4.17	178%

The table above shows how students rated their knowledge on a variety of topics before and after completing the unit. The scale used is 1 = very little knowledge, 5 = a lot of knowledge The data was obtained and compiled during the Fall 1995 semester.

Table 2
Before and after comparison

BEFORE	AFTER
forensic entomology	forensic anthropology
forensic anthropology	forensic entomology
stereo microscopy	stereo microscopy
fiber evidence	arson
water based ink dyes	DNA evidence
ball point ink dyes	thin layer chromatography
lipstick dyes	water based ink dyes
document evaluation	drug categorization
hair evidence	document evaluation
thin layer chromatography	drug laws
paper chromatography	fiber evidence
chromatography (general)	ball point ink dyes
crime scene procedures	lipstick dyes
fingerprinting	blood and body fluids
arson	chromatography (general)
drug categorization	paper chromatography
blood and body fluids	hair evidence
DNA evidence	light microscopy
drug laws	crime scene procedures
light microscopy	fingerprinting

Table 2 lists the categories in order of least knowledge to most knowledge about the topics both before and after the unit. For example before the semester the students rated their knowledge lowest in the category of forensic entomology and highest in light microscopy. After the semester they rated their knowledge lowest in forensic anthropology and highest in fingerprinting. The data from Table 1 was used to make this table.

Table 3
Percent change

TOPIC	% CHANGE
drug laws	51%
DNA evidence	61%
light microscopy	61%
drug categorization	62%
blood and body fluids	67%
arson	70%
fingerprinting	108%
crime scene procedures	120%
forensic anthropology	134%
stereo microscopy	143%
paper chromatography	144%
forensic entomology	154%
thin layer chromatography	156%
chromatography (general)	166%
water based ink dyes	176%
document evidence	178%
hair evidence	180%
lipstick dyes	188%
ball point dyes	188%
fiber evidence	194%

Table 3 shows the percent change in knowledge after the completion of the unit listed in order from least to most change. The data from Table 1 was used to make this table.

From this analysis I determined that the students feel that they come to the class with their greatest knowledge in light microscopy, drug laws and DNA evidence. The students do use the light microscope in other required science courses and they feel confident with this knowledge. In addition, I feel that the increased drug awareness programs in our school gives them exposure to the current drug laws. The class (Fall 1995) which was surveyed coincided with the conclusion of the O.J. Simpson murder trial. The students had a lot of exposure to DNA due to the trial publicity.

The three categories in which the students have the least knowledge both before and after taking the class are forensic entomology, forensic anthropology, and stereo microscopy. The only exposure they get regarding forensic entomology and forensic anthropology is from the detective who speaks to the class. The stereo microscope is used for only one lab and could explain this lack of confidence with it.

In terms of percent change the students felt they learned the most from fiber evidence, ball point dye analysis and lipstick dye analysis. These were ranked by them very low as to pre-class knowledge. The lowest percent change occurred with light microscopy, DNA evidence and drug laws. Again this coincided with the three areas they felt most confident in when coming to class.

Comments that I have heard from alumni who have gone onto college include:

"I was the only one in my Chemistry lab who knew how to use the Spec 20."

(Spring 1993)

"We had to do thin layer chromatography in our chemistry lab. Not many other students knew how." (Spring 1994)

"My college professor wants more information on your class. He thinks its great and is envious that you get to teach such a fun class." (Spring 1993)

"I've signed up for criminalistics as a career. Thanks for all you did." (Fall 1995)

DISCUSSIONS AND CONCLUSIONS

COURSE CONTENT

HISTORICAL PERSPECTIVE

As expected, the course has changed in many ways over the four years (seven semesters) that I have been teaching it. New labs have been added and other labs have been eliminated. In addition, writing assignments have been incorporated and lecture topics increased.

The first time I taught the course was in the Spring of 1993. This was prior to my thesis research which was in the summer of 1993. Therefore, I had not had the opportunity to thoroughly investigate the topic. Dr. Siegel had given me several topics and a good start but the semester was far from where it is today. Lab activities that first semester included hair and fiber analysis (though they only included microscopic examination), shoeprints, blood analysis for salicylate content (SPEC 20), fingerprinting, chromatography, and DNA labs. The labs took about twice as long as they currently do to complete due to my inexperience. Lecture topics were more limited also. I spent more time discussing the crime scene and less time discussing physical evidence. Arson, questioned documents, forensic entomology and forensic anthropology were not discussed. The students during that first semester did not have to complete any writing assignments besided their notebooks. In some ways, the students learned more, this semester, simply because they were learning while I was learning. They would help me figure out ways to make a lab better (covering the Spec 20 tubes with foil if they needed to be stored overnight) or to

decide that this activity just isn't going to work (hair perming). In addition, they gave me ideas as to areas of forensic science which they found to be most exciting (fingerprinting).

The summer following this first semester was my research summer. I got the chance to re-design the course. Changes were made in every aspect of the course. Labs, lecture topics and writing assignments were expanded.

I have found that high school students do not get enough experience writing research papers for non-English classes. Therefore, I incorporated a research paper into the course. The students are required to write a three to five page research paper on a type of evidence using at least three sources. Upon completion they are required to do a class presentation about their type of evidence. This activity has been a positive addition to the course. The students are able to incorporate their own talents into the project and then "teach" the topic to their peers. I have had students make videotapes and posters and role plays as part of their presentations. Other students have contacted experts in their topic areas and brought them in to speak to the class. This activity has promoted responsibility and creativity.

Other writing activities that I have tried have not been as successful. One group of students was asked to write a murder mystery. They were required to incorporate a minimum of three pieces of physical evidence and other guidelines were given. However, it was difficult to get a good project out of all of them. Some students just don't have the talent to be creative writers. Another writing activity included reading a mystery novel and writing a paper about the evidence used in the novel to solve the mystery. The students, in general, liked this activity but it was difficult to find mysteries which did not include a lot of sexual and violent material. The last type of writing assignment which I am still using but which needs re-designing is a current events project. The students are required to find

ten current event articles related to crime and physical evidence and write a one page evaluation of each. The activity, however, is not structured enough and many students wait until the last week to find all ten. They usually do not do a good job when they procrastinate like this. I am continuing to work on this activity and plan to use it again.

Lab activities which have been added and/or changed are many. I have tried several different hair analysis labs and have found some to be successful and others not so successful. In general, microscopic examination of hair strands and hair casts work well. However, some techniques just don't work. One hair lab called for the student to "perm" hair strands and evaluate what perm solution does to the hair strand. The lab itself worked but we were left wondering what the real purpose was. I haven't used the lab again. Fiber labs, like hair labs, are fairly simple to incorporate and there are a lot of activities dealing with fibers. Over the years, I have tried water retention activities, analysis of knit structure and fiber content labs but eliminated them due to time constraints. Document analysis labs were added the second year and have been a positive addition to the course. I plan to keep adding and changing the labs as new ideas become available.

Lecture topics are continually increasing and changing also. This last semester was the first time I lectured on forensic entomology, forensic anthropology and drug categorizaiton. In addition I've had an arson investigator as a guest speaker during the last three semesters.

The class content has improved since the Spring of 1993. This content improvement is positively reflected in the students knowledge (evaluated during class discussions), lab journal and writing activities. Discussions at the conclusion of the course include more detailed scientific debates and less personal feelings. The students develop an appreciation of the fact that the forensic scientist must remain impartial at all times.

They are aware that the evidence must be sufficient to prove guilt and that their personal bias cannot play a role in the solution. This type of mental growth is not developed in many traditional courses.

DISCUSSIONS AND CONCLUSIONS

COURSE CONTENT

THE BEST AND THE WORST

Overall, I believe that the class has been very beneficial for my students. The relaxed atmosphere taught them that they can enjoy working at their own pace and the consequences of procrastination. In addition, they've learned that working as a team means equal work for all and if one member is ineffective the whole project is jeopardized. They have learned to deal with the above situation in a responsible and polite manner.

The crime scene role play is probably the most effective part of the unit in terms of applying what they have learned. This would include their knowledge of preserving and protecting the scene, performing a thorough search and maintaining a chain of custody. In addition, they use their knowledge of physical evidence to investigate the evidence they obtain. It portrays to them the real life situation of the "unknown", also. The crime solvers go into the crime scene having absolutely no knowledge of the scenario and must investigate, analyze and interrogate until they come up with an answer. They must control their emotions no matter how frustrated they get. Finally they must work together and use the tools they have obtained in class and their higher level thinking skills to solve the crime.

The most applicable lab exercises for them are the microscope labs, the thin layer chromatography labs, the spectrophotometry labs and the electrophoresis labs. All of the

above will be used by the student in college science classes. A strong knowledge of their use will be beneficial for the student.

Least effective labs, in terms of their vague results, were the lipstick chromatography and the ouchterlony lab. The students seem to have trouble understanding the results of the lab and evaluating their uses.

The primary area, in the course, which I would like to see improved include class length. If the class became a year long course instead of a semester I would be able to teach many more topics including paint chip analysis, serial number restoration and glass and soil analysis. I would like to be able to incorporate a unit on the ethics involved with all of the technological advances, especially DNA fingerprinting.

Overall, I have truly enjoyed teaching this course. Seniors are a very enjoyable group of students to work with. They have wonderful senses of humor. They also have the maturity to be trusted with the less formal atmosphere of the course.

DISCUSSIONS AND CONCLUSIONS

COURSE CONTENT

THE STUDENTS

The majority of the students who take this course do so for one of four reasons. The first (about 20% of the students) is because they have an interest in the law and/or crime and are interested in learning more about criminal evidence. This student is not necessarily the top academic student in his/her class. The second reason (about 40% of the students) is because the student enjoys all forms of science and wants to take any and all science courses offered. This student usually is academically a good student. The third reason (about 30% of the students) is because they have had me as a teacher and want to take another course which I teach. The fourth reason (about 10% of the students) is because they have heard that the class does not have a book and there are not many tests and it is their senior year after all .... This student is usually a challenge. All students however come out of the class with an increased level of knowledge and responsibility.

Forensic science topics are usually interesting and intriguing and it is easy to get most of the students involved in discussions. I try to teach each topic by bringing in a crime where the particular type of evidence is used. Students have commented that they learn material in this course because I "sneak" it in.

Lab activities are a little more difficult to get all students involved in. I have to be alert all the time to keep all students on task. The relaxed atmosphere of the course promotes student responsibility. Some students are not ready for this responsibility and

need extra reinforcement. As the semester progresses, however, they usually progress also. I am very critical of their lab write-ups initially and many students have to re-do sketches, questions, and entire labs until they are acceptable by me. They learn quickly to take their time and do a good job initially and they will be more successful. Lab journals and lab questions are much more detailed at the end of the semester than at the beginning.

Overall, I see student growth in many areas throughout the semester. The students learn that it is alright to disagree and debate ideas with their peers. They develop an understanding of the scientific detail that is required not only in forensics but in research in general. They learn also that "less structure" means "more responsibility". Many of the students who take the course are college bound and this experience is a good preparation for them.

In general, throughout a semester, I see an increase in laboratory skills, thinking skills and student responsibility. The students learn many of these skills not by lecture but by promoting their own thinking and therefore understanding.

APPENDICES

APPENDIX A

BACKGROUND LECTURE INFORMATION

Section I : The Crime Scene

Section II: The Microscope

Section III: Chromatography

Section IV: Spectrophotometry

Section V: Electrophoresis

Section VI: Hair and Fiber Evidence

Section VII: Documents and Handwriting Analysis

Section VIII: Fingerprints

Section IX: Drugs

Section X: Toxicology

Section XI: Blood

Section XII: Deoxyribonucleic Acid - DNA

THE CRIME SCENE

In teaching a forensic unit it is not only important to teach the scientific components of evidence and instrumentation but to include a complete explanation of the crime scene. "Forensic science begins at the crime scene. If the investigator cannot recognize physical evidence or cannot properly preserve it for laboratory examination, no amount of sophisticated laboratory instrumentation or technical expertise can salvage the situation" (Saferstein 29). Retrieval of evidence from a crime scene, though not necessarily difficult needs to be done in a systematic and appropriate manner in order to preserve and protect all of the evidence.

The first officer to arrive at the crime scene has three main responsibilities. First the officer need to provide medical assistance for the injured person(s) and/or arrest the perpetrator. Secondly, the officer must eliminate any unauthorized individuals from the scene. Lastly, the officer must barricade and/or guard the area if necessary.

After the initial Preserve and Protect phase of the crime scene is complete the investigation begins. Investigators will only have a limited amount of time to survey the crime scene and therefore recording the scene becomes very important. Three general processes are involved in recording the scene. The first, photography, must take place before any of the evidence is moved or removed from the scene, with the exception of the injured parties. All physical evidence needs to be photographed. If the size of the object is significant a ruler may be placed next to it. Videotapes are acceptable. Upon completion of photography, a rough sketch of the scene needs to be done. Objects need to be shown in

the sketch by distance from two locations. Distances should be measured not guessed. North-South direction should be shown on the sketch. A final sketch will then need to be completed at the laboratory. The third record of the scene is the notes. Notes are taken throughout crime scene investigations. These are a detailed description of the investigation. Included in the notes must be a description of the scene, evidence and disposition of the evidence. "The note taker has to keep in mind that this written record may be the only source of information for refreshing one's memory months, perhaps years, after a crime has been processed. The notes must be sufficiently detailed to anticipate this need" (Saferstein 32). After the initial recording of the crime scene is completed a systematic search for evidence must begin.

A thorough and systematic search is necessary to ensure that all evidence is located. Four types of searches could be used and the specific type is determined based on the size and location of the scene. Items and methods used are determined by the investigators. For example, a typical murder scene would include a search for a weapon, possible trace evidence such as hairs, fibers or blood and any other items which might have resulted from a contact between the victim and the assailant. Failure to perform a thorough search could result in charges of "cover-up". The investigation can continue beyond the crime scene, also. Evidence obtained at an autopsy is also important in crime scene investigations. Autopsy evidence might include fingernail scrapings, blood, hair samples from the body and recovered bullets. In addition to recovering evidence controls should be obtained also.

Packaging crime scene evidence is also extremely important. Care must be taken to prevent loss, breakage, spoilage, evaporation or contamination. Every item must be packaged in it's own container and properly labeled. The investigator must be aware of the proper container needed for each type of evidence. For example, blood stained material

will mold if stored in an airtight container and should be placed in a paper bag. Charred debris, however, must be stored in an airtight container to prevent evaporation of petroleum residues. After packaging, the evidence must be submitted to the laboratory and proper chain of custody must be followed.

> Maintaining a chain of custody is extremely important for presentation in court.
>
> Adherence to standard procedures in recording the location of evidence, marking it for identification and properly completing evidence submission forms for laboratory analysis are the best guarantee that the evidence will withstand inquiries of what happened to it from the time of its finding to its presentation in court (Saferstein 38).

In many cases all individuals involved in handling evidence are asked to testify in court. It is therefore extremely important that a chain of custody is maintained.

Upon receipt of physical evidence the process of identification must begin. The expert must follow the identification procedures which have been established for that particular type of substance. Testing needs to be sufficient to exclude all other possibilities of identification. Control comparisons need to be complete in an attempt to determine a common origin.

Evidence can then be divided into two categories, class or individual. Class evidence is evidence which can be matched to a specific group but not to a type. ABO blood typing is an example of this. A blood specimen which types out as "AB" could belong to 43% of the population (Saferstein 324). This is sufficient to exclude the other 57% of the population but it does not pinpoint one individual. Individual evidence is that evidence which can be matched directly to a source. Fingerprints belong to this category of evidence. "The French scientist Victor Balthazard has mathematically determined that

the probability of two individuals having the same fingerprints is one out of 1×10^{60} " (Saferstein 365). Therefore, Forensic Science considers fingerprints individual evidence. Both types of evidence are important in the solution of crimes. Class evidence, however needs to be used in conjunction with eye witness accounts and other evidence.

THE MICROSCOPE

The microscope is probably the most valuable tool for the forensic scientist. As shown in the Lindbergh case the microscope can be the main instrument used in an investigation. From the simplest microscope, the magnifying glass, to the powerful scanning electron microscope the usefulness is extraordinary. The compound microscope is one that is commonly found in high school laboratories with magnifications ranging from 40X to 450X. The stereomicroscope is also commonly used in high schools. This instrument gives a three dimensional image with lower magnification. The comparison microscope can be found in forensic laboratories and is extremely useful in comparing two pieces of evidence or a piece of evidence and a control. This microscope basically is two separate compound microscopes which are connected by an optical bar and has one binocular eyepiece. The experimenter is able to view both stages at the same time in an attempt to compare and possibly match evidence. The fourth type of microscope used in forensics is the polarizing microscope. Glass and soil are two substances commonly analyzed with this microscope. The fifth type, only used in advanced laboratories, is the Scanning Electron Microscope. Instead of using light as an imaging source the microscope utilizes electron emission. The SEM image has a high magnification and resolution.

CHROMATOGRAPHY

The second important type of forensic analysis is chromatography. There are several types of chromatography. The basis behind all types however is the same. "The theory of chromatography has as its basis the observation that chemical substances have a tendency to partially escape into the surrounding environment when dissolved in a liquid or when absorbed on a solid surface" (Saferstein 106). In general, chromatography is said to have a stationary phase and a moving phase. Sample components which have a chemical preference for the stationary phase will not travel as far on a chromatogram as those that prefer the moving phase. The two types of chromatography used in the high school classroom are paper chromatography and thin layer chromatography. Paper chromatography utilizes paper as its stationary phase (Modern Chemistry Laboratory Experiments 43) and usually water as one choice of solvent. In thin layer chromatography a glass plate usually is coated with silica gel or aluminum oxide as its stationary phase. A solvent (moving phase) is drawn through this granular phase by means of capillary action. "Those components with the greatest affinity for the moving phase will travel up the plate at a faster speed as compared to those that have greater affinity for the stationary phase" (Saferstein 113). Upon completion of the chromatography the plate is visualized. This sometimes requires the use of ultraviolet light. An Rf measurement is then taken of all of the bands. More advanced types of chromatography are used in forensic laboratories. These include Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC). Gas chromatography uses gas as its moving phase. This gas flows over a thin

film of liquid which is the stationary phase. In HPLC the "moving phase is a liquid which is pumped under pressure through a column filled with fine solid particles" (Saferstein 112). This process is different from GC in that it can be performed at room temperature. Therefore, heat sensitive substances can be analyzed without destruction. Organic explosives and some types of drugs are commonly analyzed using HPLC. The major drawback with chromatography techniques is that the analysis is not conclusive.

SPECTROPHOTOMETRY

Spectrophotometry is another form of analysis used in evidence identification. This technique utilizes the absorption of light by a substance as its basis. Substances can be categorized by the light they absorb. The spectrophotometer measures this absorption. As light is passed through the sample, the spectrophotometer detects and records the overall light absorption by the substance at a given wavelength. The typical spectrophotometer used in a high school utilizes an ordinary tungsten light bulb as its light source. More advanced laboratories use ultraviolet, infrared or mass spectrophotometers. Ultraviolet spectrophotometers measure the absorbance of ultraviolet light. Infrared spectroscopy measures infrared absorption and is much more sophisticated than ultraviolet due to the complexity of infrared light. The infrared spectrum of a substance is considered a "fingerprint" of that substance. The mass spectrophotometer is used in conjunction with the GC as a means of producing a specific identification. The mass spectrophotometer fragments and then masses the components of the sample which emerge from the GC. No two substances have the same fragmentation pattern.

ELECTROPHORESIS

Electrophoresis is another useful procedure used in evidence identification. This process is similar to thin layer chromatography except that electricity is used to separate the substance components. The stationary phase is usually a gel like substance which has wells in it. The samples are placed in the wells and an electric current applied to the gel. Components with an electric charge will migrate in the gel. This procedure is used extensively with blood and blood component identification.

HAIR AND FIBER EVIDENCE

Hair and fibers are often left at crime scenes. "Every time we enter a room, we deposit a bit of ourselves. When we leave, we take something with us" (Nichols 22). This causes even the most perfect crime to have flaws. Hair and textile fibers are left by even the best criminal. Both types of evidence fall into the category of class evidence. It is possible to determine a group of individuals to whom the evidence might belong to but rarely one specific item or individual. An exception to this might be a large piece of fabric which was torn out of a piece of clothing and which can be matched back to that item. There are many characteristics of both that make them useful in criminal investigations.

Hair is a protein substance which grows out of an organ known as a hair follicle. "The average, normal human body has approximately 250,000 hairs. These hairs are normally shed at the rate of 250 per 24-hour period" (Miller and Brown 83). There are three major parts to the shaft of the hair, the cuticle, cortex and medulla. The cuticle is the outermost or external part of the hair. It is composed of a series of overlapping scales. The microscopic appearance of these hairs is called a scale pattern. Scale patterns are very useful in determining the species of animal that the hair is from. See Figure 1. Inside the cuticle is a region called the cortex. The cortex contains the pigment granules of the hair shaft. These granules and their distribution are useful in determining the racial origin of a human hair. The central portion of the hair shaft is known as the medulla. There are four general categories of medullas: they are fragmented, interrupted, continuous or stacked. The type of medulla present in a hair shaft helps to determine the species of animal which

the hair belonged to. See Figure 2. Other characteristics of the hair which are useful in identification include color, length, diameter, cross section (See Figure 3), root patterns (See Figure 4) and medullary index. The medullary index is the ratio of the medulla diameter to the shaft diameter.

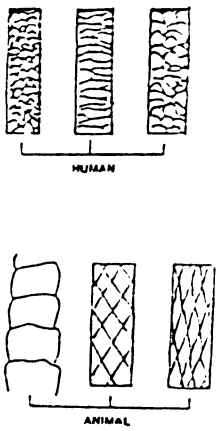

Figure 1: Scale patterns.

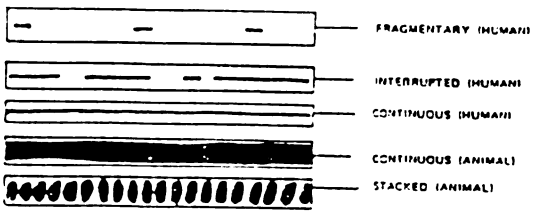

Figure 2: Medullas

Miller, L.S. and A.M. Brown. (1990). Criminal Evidence Laboratory Manual An Introduction to the Crime Laboratory Second Edition. Ohio. Anderson Publishing Company.

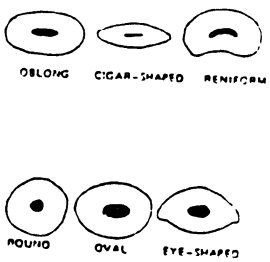

Figure 3: Cross sections

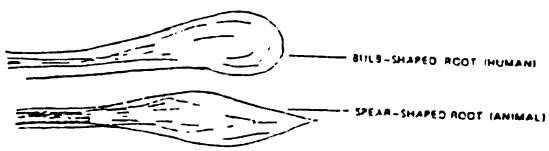

Figure: Root patterns

Miller, L.S. and A.M. Brown. (1990). Criminal Evidence Laboratory Manual An Introduction to the Crime Laboratory Second Edition. Ohio. Anderson Publishing Company.

Textile fibers, like hair (sometimes they are hair), are commonly found at crime scenes. Fibers can be divided into two categories, natural and man-made. "There are three basic natural fibers from which clothing and material are made: (1) silk, (2) wool, and (3) cotton" (Miller and Brown 169). There are two types of man-made fibers, regenerated and synthetic. Regenerated fibers are which are produced from the cellulose components of natural raw materials. Examples include rayon and acetate. Synthetic fibers are those which are manufactured totally from synthetic materials. Examples are nylon and polyester. Fiber characteristics which are useful in identification include microscopic appearance (color, diameter and cross section), effect on litmus, residue and odor from burning, birefringence and solubility.

DOCUMENTS AND HANDWRITING ANALYSIS

Documents and handwriting are another type of evidence sometimes left at crime scenes. Questioned documents can be either class or individual evidence depending on the situation. Any document with handwritten or typed markings on it and whose authenticity is in doubt are considered questioned documents. Many items fall under this broad definition. Letters, checks, lottery tickets and wills are examples. Other items which might be considered "questioned documents" are walls, windows and doors. Analysis of documents includes determining chemical make-up such as fiber content and ink analysis. In addition attempts to alter a document must be recognized by the document examiner. Handwriting comparisons are another type of analysis the document examiner performs. Unconscious writing can never be duplicated between two individuals. Characteristics which make handwriting samples different include line quality (the skill of writing), spacing of words and letters, letter size, penlifts and separations, connecting strokes, beginning and ending strokes, unusual letter formations, shading, slant, baseline habits, embellishments and placement of diacritics (Miller and Brown 201). Comparisons must include both those characteristics which are the same between two writing samples and an explanation of any characteristics which are different. A sufficient number of comparisons must be made between a known and questioned document to exclude the possibility that the document originated from any other source. Therefore handwriting is considered an individual type of evidence.

FINGERPRINTS

Fingerprint evidence is especially interesting to discuss in the high school classroom because of the widespread use in crime related television shows and movies. "In the United States, the first systematic and official use of fingerprints for personal identification was adopted by the New York City Civil Service Commission in 1901" (Saferstein 365). Fingerprint evidence is now widely used and accepted as individual evidence.

There are three fundamental principles of fingerprinting. The first states that a fingerprint is individual evidence. In a 90 year period no two fingerprints have been found to be identical. The second principle states that a fingerprint will not change during an individuals lifetime. Attempts at scarring the skin to obliterate a print have been shown to be unsuccessful. The third principle states that fingerprints result from skin ridges on the surface of the palms of the hands and soles of the feet. On these ridges are pores. Sweat and oils are released from the skin through these pores. Whenever an individual touches an object traces of these oils are left behind as a "latent" fingerprint. These prints can then be dusted with a fine powder, lifted with clear tape and deposited onto a contrasting material. Once successfully lifted a classification can begin.

Classification of a print requires analysis of the ridge characteristics of the print. Three general categories of fingerprint types exist: loops, whorls and arches. Loops are the most common type of print. "A loop must have one or more ridges entering from one side of the print; recurving, and exiting from the same side" (Saferstein 369). See Figure 5.

There are two types of loops, an ulnar loop and a radial loop. Ulnar loops open towards the little finger while radial loops open towards the thumb. The second type of fingerprint is the whorl. There are four categories of whorls; plain, central pocket, double loop and accidental. Plain and central pocket whorls each contain one ridge which makes a complete circuit. "If an imaginary line is drawn between the two deltas (See Figure 7) contained within these two patterns, and if the line touches any one of the spiral ridges, the pattern is a plain whorl. If no such ridge is touched, the pattern is a central pocket loop" (Saferstein 371). A double loop is simply "two loops combined into one fingerprint" and an accidental whorl "either contains two or more patterns (not including the plain arch) or is a pattern not covered by other categories" (Saferstein 371). The last type, arches, are characterized by ridges entering one side of the print and exiting the other. Two categories of arches exist. Plain arches rise smoothly in the center while tented arches have a distinct spike in the center (Saferstein 371). See Figure 5. "Sixty to 65 percent of the population has loops, 30 to 35 percent has whorls and about 5 percent has arches" (Saferstein 369).

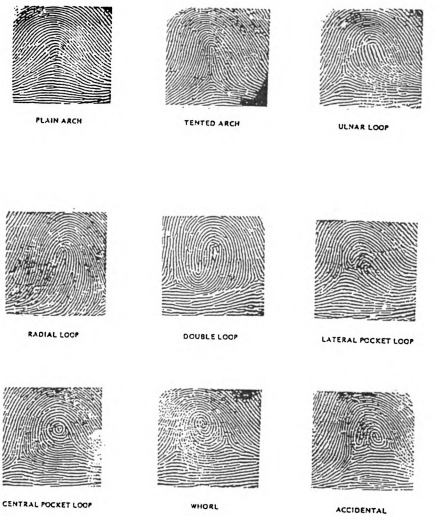

Figure 5: Fingerprint types.

Miller, L.S. and A.M. Brown. (1990). Criminal Evidence Laboratory Manual An Introduction to the Crime Laboratory Second Edition. Ohio. Anderson Publishing Company.

After initial determination of a print type a more detailed analysis can begin. There are several points of comparison which can be made between two prints. Included in these points are "ridge endings, bifurcations, islands, dots, bridges, spurs, enclosures, double bifurcations, deltas and trifurcations" (Miller and Brown 31).

> Criminal courts will accept a minimum of twelve identical "characters" or "points" as a positive match in latent print identification and comparison with known inked prints. When twelve identical characters are located and properly marked, the identity of the suspect cannot be refuted (Miller and Brown 31).

Besides comparison of individual points a classification of a complete set of ten prints has been established.

The first fingerprint classification system, known as the Henry system, was developed in 1901 by Scotland Yard. This system, however, has been replaced in the United States by the FBI system because of the need for an expanded system. This new system is used to determine the primary classification of a set of prints. The presence of a whorl on a given finger is the determination of the numerical value for that finger. Once this value is determined it is placed in the fractions below.

R. Index	R. Ring	L. Thumb	L. Middle	L. Little
R. Thumb	R. Middle	R. Little	L. Index	L. Ring

A whorl in fraction 1 gets a value of 16; fraction 2, a value of 8; fraction 3, a value of 4; fraction 4, a value of 2; and fraction 5, a value of 1. All individuals then add 1/1 to their ratio. If a whorl is not present on a given print then that finger gets a value of 0.

For example, Sue O. has whorls present on her left thumb, right thumb and right middle. Her classification would look like this:

$$\begin{array}{llll} \underline{0 + 0 + 4 + 0 + 0 + 1} & & & \underline{5} \\ 16 + 8 + 0 + 0 + 0 + 1 & & & 25 \end{array}$$

To use this system a complete set of prints needs to be obtained and rarely are all 10 prints left at a crime scene. Another system has been developed to deal with this problem.

Automated Fingerprint Identification Systems (AFIS) are computer scanning devises which "digitally encode fingerprints so that they can be subject to high speed computer processing" (Saferstein 373). This system has allowed an integration of fingerprints across the nation. Individuals who are arrested for a crime are fingerprinted and these prints are entered into the AFIS system. If this individual has left prints at a crime scene somewhere else in the United States the system will indicate this. The major advantage of the system is the time it saves investigators. Much less time is being spent developing suspect lists and more time spent investigating suspects generated by the AFIS computer system.

DRUGS

Drugs and their analysis play a large part in many criminal investigations. "In the United States, the epidemic proportions of illegal drug use has produced a situation that finds more than 75 percent of the evidence being evaluated in crime laboratories to be drug related" (Saferstein 216-217). Drugs fall into two major categories, illegal and legal. There are four categories of commonly abused drugs; depressants, stimulants, narcotics and hallucinogens. Narcotics are those drugs which relieve pain by acting on the Central Nervous System. Examples of narcotics are morphine and heroine. Depressants, in general, slow down and impair normal body functions. Alcohol and barbiturates are both depressants. Stimulants are drugs which produce a feeling of alertness followed by a decrease in fatigue and appetite. Cocaine and amphetamines are both stimulants. Hallucinogens are drugs that cause changes in normal thought processes. Examples of hallucinogens are marijuana and LSD. In order to have uniform drug laws throughout the United States, the federal government has established the Uniform Controlled Substances Act. "The federal law establishes five schedules of classification for controlled substances on the basis of a drug's potential for abuse, potential for physical and psychological dependence, and medical value" (Saferstein 234) . Schedule I drugs have a high potential for abuse and have no currently accepted medical use. Included in this schedule are heroin and marijuana. A schedule II drug is one which has a high potential for abuse but does have a current medical use. Schedule II drugs include PCP and amphetamines. Schedule III drugs have a lower potential for abuse and have a currently accepted medical use.

Codeine falls into this category. Schedule IV drugs have a low potential for abuse and have a current medical use. Darvon and Valium are both schedule IV drugs. Schedule V drugs have low potential for abuse. The criminal penalties for sale and possession of drugs is related to their placement in the schedule. The lower the schedule the higher the penalty. Several types of testing exist for identifying drugs but because of the evidence few can be used in a high school laboratory.

TOXICOLOGY

Forensic toxicology is another type of investigation that is closely related to drug analysis. Toxicology involves the detection of drugs and poisons in blood or body fluids, tissues or organs. Alcohol is a major category of drug that the toxicologist looks for. The toxicologist, however, is also responsible for identification of other drugs and poisons. Once identification of the drug is complete the toxicologist may then be called upon to assess its influence on the behavior of the individual involved.

BLOOD

Blood samples have other applications at the crime scene also. Upon obtaining a blood stain a forensic serologist must determine whether or not the source of the blood is human. If so, an initial blood typing will be done. Common tests include A-B-O typing and Rh factor typing. The basis for both tests is that a red blood cell contains on its surface a protein structure called an antigen. An individual with type A blood has "A" antigens on the surface of their red blood cells, individuals with type B blood have "B" antigens on their red blood cell surface, and individuals with type O blood have neither antigen on the surface of their red blood cells. For every antigen there is a specific antibody. If the antibody for B type blood is given to an individual with type B blood agglutination will occur. This massive clumping of red blood cells in the human body could cause death due to the inability of the red blood cell to do its primary job of transporting oxygen and carbon dioxide. The procedure of typing blood is simply a process of placing the unknown blood sample into samples of known antibodies and watching for agglutination. Rh typing follows the same principle. An Rh positive person has the Rh antigen while the Rh negative person does not. The entire process of blood typing is used as class evidence and is most useful in eliminating suspects. A typical distribution of blood types in the United States is 43% type O, 42% type A, 12% type B and 3% type AB.

DEOXYRIBONUCLEIC ACID - DNA

Deoxyribonucleic acid (DNA) is the name given to the genetic material found in most cells of living organisms. The coding of certain parts of DNA is unique to every individual. These unique parts can be used to verify to whom a sample of blood or body fluid belongs. "Sample evidence taken from a crime scene when matched with a suspect's DNA fingerprints, can be used to prove innocence or guilt" (Science Kit and Boreal Laboratories 1). Human genes are the subunits of DNA. The nucleotide base sequence related to a certain gene determines the characteristics that an individual will have. This gene coding is similar between all humans. Between these codes for genes, however, are the random codes that have become important to forensic scientists. "Not all the letter sequences in DNA code for the production of proteins. Portions of the DNA molecule contain sequences of letters that are repeated several times" (Saferstein 353). It is these repeating strands, called restriction fragment length polymorphisms (RFLP's) that are used to distinguish one individual from another.

The process of DNA typing follows the following sequence. The DNA is isolated from the blood or body fluid sample. Restriction enzymes are added to the sample. These enzymes are specific for known or target codes of DNA. The restriction enzymes cut the RFLP's out of the DNA sample. The RFLP's are then placed into a gel electrophoresis apparatus. An electric current is applied to the gel. Since DNA is a negatively charged molecule it will migrate towards the positive pole. The smaller DNA fragments will migrate further in the gel than the larger fragments. I like to use this analogy with my

students. Imagine a bowl of jello with cut up peaches in it. Some of the pieces are very large and some are very tiny. Imagine then that you put a straw into the jello and try to suck the cut up peaches up to the straw. The smaller pieces will be easier to pull through the jello than the larger pieces. When electrophoresis is complete a radioactive probe with a complementary base sequence is attached to the DNA sample. The DNA can now be seen using X-rays. This "fingerprint" can be used to compare a suspects DNA to DNA found at a crime scene. Many cases have been solved using DNA fingerprinting. In addition people have been released from prison because DNA fingerprinting showed them to be innocent.

APPENDIX B

LABORATORY ACTIVITIES

Section I: Paper Chromatography

Section II: Practice in the Use of the Microscope

Section III: Examination of Hair by Light Microscopy

Section IV: Analysis of Fibers

Section V: Carpet Sample Lab

Section VI: Determination of Lipstick Dyes Using TLC

Section VII: The Analysis of Questioned Documents

Section VIII: Analysis of Inks By Thin Layer Chromatography

Section IX: Visualization and Examination of Fingerprints

Section X: Shoeprints

Section XI: Urine Analysis Using Paper Chromatography

Section XII: Ouchterlony Diffusion

Section XIII: Determination of the Peak Absorbance of a Substance Using Visible Spectrophotometry

Section XIV: Identification of Salicylates In Blood By Spectroscopy

Section XV: The Alcohol Breathalyzer Test: A Laboratory Simulation

Section XVI: Agarose Gel Electrophoresis

Section XVII: DNA Electrophoresis

PAPER CHROMATOGRAPHY

Paper chromatography is a method of separating mixtures by using a piece of absorbent paper. In this process, the solution to be separated is placed on a piece of dry filter paper. This is the stationary phase. A solvent (the moving phase) is allowed to travel across the paper by capillary action. As the solvent front moves, the components of the mixture separate. The components of the mixture that are most soluble in the solvent and least attracted to the paper travel the farthest.

In this experiment you will place a small spot of black, water soluble ink near the center of a piece of filter paper. By means of a wick, water will be drawn to the center of the filter paper and will then spread outward towards its edge. The colored molecules that make up the black ink mixture will be distributed by the solvent along a path to the edge of the paper. Because each different brand of black ink is a unique mixture of colored molecules, each pattern on the paper or chromatogram is characteristic of the brand of pen used.

Chromatography is an important tool of the forensic chemist in solving crimes. This method can help identify the brand of pen used to write a ransom note. Using chromatography, ink manufacturers can quickly determine if a competitor has stolen their "secret" formula.

OBJECTIVES: At the completion of this laboratory exercise the student will be able to:

1. Perform the technique of paper chromatography.
2. Separate the colors of different inks.
3. Identify an unknown ink using paper chromatography.

CRIME SCENE:

Upon arrival to school today, Mrs. Pawloski found a threatening note attached to her door,... "Chemistry II students, BEWARE, your lives are in danger. You will be sorry for treating me so poorly." After careful evaluation Mrs. Pawloski narrowed her list of suspects to six. She then confiscated their black ink pens. Your job is to analyze the inks by paper chromatography and determine who the culprit is.

MATERIALS:

filter paper, 12 cm
six numbered, water soluble, black ink pens
filter paper wick
petri dish
filter paper with four spots of unknown black ink
sample of ink from threatening note

PROCEDURE 1: Paper chromatography

1. Use a pencil to sketch a circle about the size of a quarter in the center of the piece of filter paper. See Figure 1.

2. On the circle make a dot with pen #1 and write a number 1 next to it with a pencil. Using black pen # 2 make a dot and place a number 2 next to it with a pencil. Repeat with all pens.

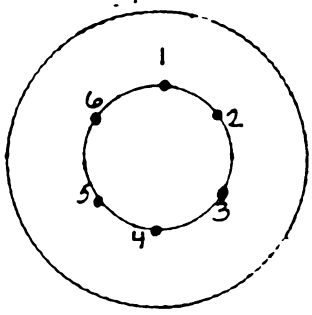

Figure 1

3. Use a pencil to poke a small hole in the center of the filter paper. Insert a piece of filter paper wick through the hole.

4. Fill the petri dish bottom halfway with water. Set the wick of filter paper into the water. **Do not allow your black dot prepared paper to become submerged in the water.** See Figure 2.

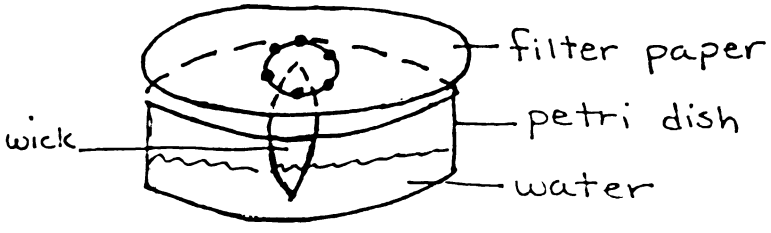

Figure 2

5. When the water is 1 cm from the outside edge of the paper, remove the paper from the petri dish and allow the chromatogram to dry. Record the colors that have separated from each of the six different black inks in the data table. You may want to use colored pencils to record this information.

6. Ask Mrs. Pawloski for a piece of filter paper with four black dots of unknown ink. Repeat steps 3 through 5.

7. Use your known information to identify your unknown inks. Record this information in your data table.

PROCEDURE II: Identification of the pen used to write the note.

1. Prepare a procedure for identifying the pen which was used to write the scandalous note.

2. Perform the procedure and determine which pen was used.

3. If the procedure was successful, write it up, step by step, on page 4 of this lab sheet. If the procedure didn't work, prepare a different one and perform it. Continue this until you get a procedure which works. Write up the correct procedure on page 4 of this lab sheet.

4. Record your pen identification conclusion in the conclusion section of the lab sheet.

DATA:

KNOWN PEN INKS

pen number	dot number	record of chromatogram center	middle	edge
1	1			
2	2			
3	3			
4	4			
5	5			
6	6			

UNKNOWN INKS

pen number	dot number	record of chromatogram center	middle	edge
7	7			
8	8			
9	9			
10	10			

INK FROM THREATENING NOTE

Record of chromatogram

center	middle	edge

CONCLUSION:

1. Which known pen made the dot of the following unknown inks?

7 - _____

8 - _____

9 - _____

10- _____

2. a. Who did you conclude wrote the threatening note?

b. Why did you conclude this? Be specific.

QUESTIONS:

1. What procedure did you use to identify the ink from the note?

2. Some components of ink are minimally attracted to the stationary phase and are very soluble in the solvent. Where are these located on the filter paper during chromatography?

3. What can be said about the properties of a component ink that travels only half the distance to the final solvent front?

4. Predict the results of forgetting to remove the chromatogram from the water in the petri dish until the next day.

REFERENCES:

Tzimpoulos, Nicholas D., H.C. Metcalfe, J. Williams, Joseph Castka. (1990). Modern Chemistry Laboratory Experiments. Teacher Edition. Texas. Holt, Rinehart, and Winston Inc.

Revised by Karen Pawloski

PRACTICE IN THE USE OF THE MICROSCOPE

The microscope is one of the most valuable tools of the forensic scientist. She uses it to study hair, fibers, seeds, soils, metals, paints - anything and everything involved in a crime. It is believed that engravers used glass globes filled with water as magnifying glasses at least 3000 years ago. The simplest microscope is called a magnifying glass. Optical microscopes magnify because light rays reflected from an object bend (refract) as they pass through one or more lenses.

How big you can make the object depends on the refractive index (bending power) of the glass in the lens. Hand lenses are 3 to 10X. Since the light rays are spread out when an object is magnified, the magnified object is not as bright as the original. To make it as bright as it was originally, additional light must be used.

Suppose that you took a small section of a magnified object and placed a second lens over it. This magnified section could then be further magnified and we would have a compound microscope. The Dutch spectacle maker, Zacharias Janssen, is credited with discovering this principle and making the first compound microscope in 1590. Since then, there have been many improvements, although the basic concept has remained essentially unchanged. Magnification up to 400X is possible with ordinary illumination. With a substage condenser to focus more light on the object and with better lenses, it is possible to go to 1000X magnification. About the highest magnification that can be obtained with a compound microscope is 2500X.

The quality of a microscope resides in the lenses. Slight imperfections can cause large distortions in the object that is viewed. In addition, the various colors refract at different angles, so correcting lenses must be added. Expensive microscopes have excellent correcting lenses, while less expensive microscopes may not even have correcting lenses.

In working with a compound microscope, you will find the following terms useful.

Working distance - the distance between the specimen and the tip of the objective lens. In general, the higher the magnification, the shorter the working distance.

Depth of focus - the thickness of the object that is simultaneously in focus. The higher the power of magnification, the less is the depth of focus.

Field of view - the area or diameter of the specimen that is in view. The higher the power of magnification, the less is the field of view.

The general structure of the compound microscope is shown in Figure 1.

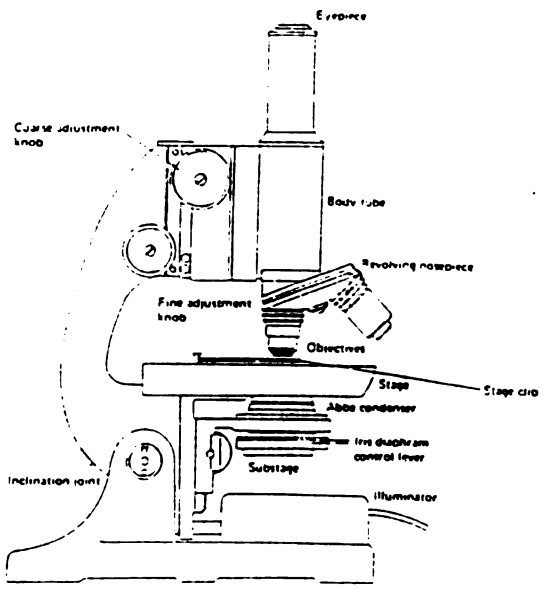

Figure 1: The Microscope

Clifton Meloan, Richard James, Richard Saferstein. (1990). Criminalistics: An Introduction to Forensic Science Lab Manual. New Jersey. Prentice Hall.

MATERIALS:

microscope, compound
medicine dropper
scissors
microscope slides
tea
unknown ink sample
microscope, stereoscopic
lens paper
cover slip
newspaper
paper match books
unknown paper match sample

PROCEDURE:

PART A:

1. Obtain a compound microscope from the cabinet, and carry it back to you lab table.

2. With a piece of lens paper, lightly wipe any dust and grease from all exposed glass surfaces. Never use anything else to do this job.

3. Spend the next few minutes becoming familiar with the names and locations of the various important parts of the instrument.

Several important rules are to be noted in connection with the foregoing procedures:

a. To find an object, always start your examination with the low power objective, never with the high. The low power reveals an area on the slide some 20 times greater than the high-power, making it 20 times easier to locate the desired object.

b. To bring the object into focus, always focus upward, with the coarse adjustment. (NOTE: Some microscopes focus by moving the stage up and down rather than the body tube. In such cases the focusing is downward- away from the moveable parts.) You want to focus upward because when your eye is at the ocular (eyepiece), it is impossible to determine how far down or up the tip of the objective has traveled. In focusing down, carelessness may result in crushing the specimens.

c. When the high power objective is being used, never use the coarse adjustment.

4. Cut the letter "h" from a newspaper. Place it on a clean slide, and with a medicine dropper, place 1 drop of water on the letter.

5. Wait a moment before covering it with a cover slide.

6. Place the slide on the stage and clamp it down. Move the slide so that the letter is in the middle of the hole in the stage. Make certain that the low-power objective is in place. Viewing the stage from the side, use the coarse adjustment wheel to lower the objective until either the stop is reached or the objective is approximately 2 cm from the cover slip.

7. Turn on the substage illuminator on the microscope.

8. Now, looking through the ocular, slowly raise the tube with the coarse adjustment knob until the letter "h" is in focus. If you cannot see the object, center the slide more carefully and repeat the whole procedure. The focus may be made sharper by a slight turn on the fine adjustment knob.

9. Open and close the iris diaphragm by turning the diaphram handle, which projects laterally from the lower portion of the condenser. The iris diaphragm controls the amount of light reaching the specimen. Adjust it to make the image as sharp as possible.

10. The image is in focus 3 to 4 mm above the eyepiece. Thus, there is no reason to press one's eye to the ocular.

11. To change to the high power, make sure that you have focused sharply under low power on the object and centered it in the field. Then carefully swing the high power objective into place. The high power objective should not strike the slide, though it will come very close. A few turns of the fine adjustment knob, either up or down, should suffice to bring the "h" into sharp focus.

12. Draw the "h" shown on high power in your lab notebook. Don't forget to carefully label your drawing.

PART B: THE STEREOSCOPIC MICROSCOPE:

1. Using the stereoscopic microscope at the front lab station, familiarize yourself with the parts of the microscope.

2. Place the previously prepared "h" slide onto the microscope stage.

3. Set the magnification knob to the highest power (2X or 3X). Look through the right eyepiece and adjust the focusing knob until the letter "h" is sharp.

4. Draw the "h" seen using the stereoscopic microscope in your lab notebook.

5. Using the steps outlined above, examine tea using the stereoscopic microscope.

6. Draw the tea seen using the stereoscopic microscope in your lab notebook.

7. Examine the tips of your fingers under the stereomicroscope. Locate the ridges that form a fingerprint. Locate the sweat pores that exist on these ridges.

8. Draw a stereoscopic image of your fingerprint in your lab notebook.

PART C: COMPARISON OF PAPER MATCHES

From time to time a forensic laboratory may be asked to see whether a torn-out paper match comes from a partially used book, usually taken from an accused person.

Cursory examination of any matchbook will reveal that it contains two pieces of cardboard secured in the book with a staple. The individual match body is formed by a series of partial cuts in this cardboard; thus each layer of matches was originally a single piece of cardboard.

The obvious first attempt to match a torn-out match to a partially filled match book requires physically fitting the torn edges of the match to the corresponding portion of the torn book. Barring success in this attempt, a forensic examiner will then try to compare the suspect match with matches remaining in the book in order to establish an adjacent relationship.

Such comparison can be conducted under a stereoscopic microscope. The most significant features to look for in the comparison of paper matches are:

1. Color, width and thickness.

2. Most matches are made from reprocessed cardboard. Examination of the match edges reveal inclusions consisting of a large variety of colored fibrous material, aluminum foil, and other contaminants that were involved in the production of the cardboard. A side-by-side examination of matches for comparable inclusions is probably the easiest and most significant feature to look for in match comparisons.

3. Another feature for comparison is the presence of continuous fibers between adjacent matches. These fibers may exist on upper and lower surfaces of the matches.

CRIME SCENE "A"

A burglary has been committed, and, apparently, in addition to the burglars inside the building, a lookout was posted. The detective at the scene noticed a few cigarette butts by the back door, as well as a few paper matches. He picked these up as possible evidence. a few days later three suspects were apprehended. A search of the clothing in their apartments yields several books of matches in their pockets. Your job is to see if the match found at the back door is from any of the books recovered.

Method

1. You will be given one match from the crime scene and the matchbooks.

2. Compare the matches and books for color, width and thickness similarities.

3. Under the highest power of the stereoscopic microscope, hold the edges of the matches side by side and compare them for any matching characteristics.

4. Determine whether your match could have originated from any of the matchbooks.

CRIME SCENE "B"

A student at a prominent University in Michigan is suspected of cheating on his Chemistry final exam. The situation actually occurred when the student was returned the graded exam. The professor who corrected the exam said that the student did not give an answer for problem #17 and the professor drew a red "X" through the box where the answer should have been. However upon reviewing the returned test the student said that there was an answer in the box and it was correct. The answer was written in pencil. Your job is to determine, using the stereoscopic microscope which was written first, the red ink pen or the pencil.

DATA:

Make sketches of everything you've looked at using the microscopes in your notebook. Make sure everything is properly labeled.

QUESTIONS:

1. When you examined the "h" under the low power objective of the compound microscope was the image right side up?

2. What is the total magnification at high power of your compound microscope?

3. When you examined the "h" using the stereoscopic microscope was the image right side up?

4. What was your conclusion for Crime Scene "A"? Explain your answer. Use diagrams and sketches.

5. What was your conclusion for Crime Scene "B"? Explain your answer. Use diagrams and sketches.

REFERENCES:

Clifton Meloan, Richard James, Richard Saferstein. (1990). Criminalistics: An Introduction to Forensic Science Lab Manual. New Jersey. Prentice Hall.

Revised by Karen Pawloski

EXAMINATION OF HAIR BY LIGHT MICROSCOPY

The analysis of single hairs is fairly straightforward, involving almost exclusively a microscopic morphological examination of the internal structure of the hair. The analysis of a suspect hair from a crime scene involves the determination of whether or not the object is a hair or a fiber, what species of animal it belongs to if it is a hair and finally, what degree of association can be made between the crime scene hair and hairs from a known source.

Although it is not possible to individualize a single hair (or even a whole group of hairs) to a particular person (or animal), it is possible to associate the unknown hair to an individual to a very high degree. This is accomplished by a careful morphological examination which will determine a number of physical characteristics. In comparing known and unknown hairs, the similarity of these characteristics will determine the degree of association of the hairs.

In this lab you will examine animal (non-human) and human hairs to determine how one species can be differentiated from another. You will then be given hairs taken from a crime scene and asked to evaluate them.

MATERIALS:

compound microscope
coverslips
horse hair
cat hair
isopropyl alcohol
rubber cement
microscope slides
human hair
dog hair
glycerin
clear nail polish
Polaroid fixative

PROCEDURE:

PART A: Visual Examination of Hair

1. Look at the hair. Document the color and length of the hair.

2. Feel the hair. Determine and document its coarseness.

3. Document the shape of the hair strand. For example, is it straight or is it curly?

PART B: Microscopic Examination of Hair

1. Obtain a strand of human head hair. This can be taken from your head or your partners head.

2. Place the hair on a microscope slide.

3. Place a drop or two of glycerin on the hair and place a coverslip on top of it. This is called a wet mount.

4. Place the slide on the stage of the compound microscope and adjust the magnification to 100X.

5. Locate the root end of the hair, if it has one. If the hair has been forcibly pulled out you will see a bulb shaped enlargement.

6. Make a sketch of what you see in your lab notebook.

7. Scan the length of the hair. Observe the medulla. If one is present, determine the type. Draw a sketch of the hair shaft in your notebook.

8. Examine the tip of the hair. If the hair has been cut recently you will to a square appearance to the end. If the hair has split ends, this is normally due to waving, bleaching or repeated brushing.

9. Evaluate the medullary index of the hair.

10. Repeat steps 1 - 8 for dog, cat and horse hair. Make careful observations to determine any microscopic differences between the four types of hair you have observed.

PART C: Scale Patterns.

Scale patterns are of little value in human hair comparisons but can aid in distinguishing animal hairs. You will now examine scale patterns of dog, cat, horse and human hair.

1. Clean a strand of human hair by pulling it through a fold of tissue moistened with alcohol to remove grease and oil from the hair surface.

2. Smear Polaroid fixative on a clean microscope slide.

3. Imbed hair into the fixative.

4. Let dry 5 - 10 minutes.

5. Remove hair from fixative.

6. Examine the scale patterns using the compound microscope.

7. Sketch what you see in your notebook.

8. Using a hair from the same source repeat steps 1 - 7 using rubber cement as your mounting medium.

9. Using a hair from the same source repeat steps 1 - 7 using nail polish as your mounting medium.

10. Repeat steps 1 - 9 for horse, cat and dog scale patterns.

PART D: Hair cross sections.

1. Obtain 2 or 3 human head hairs.

2. Thread them 2 - 3 cm up the tip of a Pasteur pipet.

3. Pour one or two drops of Norlands 65 into the pipet from the top and allow to run into the tip.

4. When the tip is full cure the Norlands under long wave UV light for 15 minutes.

5. With a single edge razor blade, chip away the glass of the tip and cut a thin section of the Norlands and hairs.

6. Mount it in a drop of 1.53 Cargille Refractive Index oil with a coverslip.

7. Observe under the microscope.

8. Sketch what you see in your notebook.

9. Repeat steps 1 - 8 for horse, cat and dog hair.

PART E: Crime scene

A women is found murdered in her apartment. Upon examination of her clothing the detectives find several strands of hair. The woman has long blond hair but the strands all range between 3 and 6 cm in length and are black in color. The woman has two dogs and three cats. You will be given the hair samples which were retrieved from the clothing. Determine the species origin of the hair samples.

FOLLOW-UP:

1. Prepare a table comparing the morphological characteristics of human, dog, cat and horse hair.

2. Explain why hair is considered class evidence.

3. Explain the solution to your crime scene evidence.

REFERENCES:

Clifton Meloan, Richard James, Richard Saferstein. (1990). Criminalistics: An Introduction to Forensic Science Lab Manual. New Jersey. Prentice Hall.

Schlitz, Gary. (1994). Forensic Laboratory Science and Detective Mystery Writing. Illinois. Flinn Scientific, Inc.

Siegel, Jay. Forensic Science Lab Manual. Michigan State University, East Lansing, Michigan. 1993.

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ANALYSIS OF FIBERS

Fibers can be very important pieces of evidence in incidents that involve personal contact - such as homicide, assault or sexual offenses. The ultimate value of a fiber depends on the ability of a criminalist to narrow the origin to few sources or even a single source. In this lab exercise you will use several techniques to identify different fibers.

MATERIALS:

Fabric samples
- cotton
- linen
- silk
- wool
- dacron
- nylon
- orlon
- acetate

6 M NH_4OH

6 M H_2SO_4

6 M HCl

Pasteur pipets

filter paper
bunsen burner
ring stand
test tube clamp
red litmus paper
blue litmus paper
test tubes 13 x 100 mm
Norlands 65 oil
1.53 Cargille Refractive Index Oil
1 large test tube

PROCEDURE:

PART A: Microscopic Examination.

1. Place 2-3 cotton fibers on a clean microscope slide.

2. Place 1-2 drops of glycerin onto the fibers.

3. Cover with a coverslip.

4. Observe using a compound microscope.

5. Sketch what you see in your lab notebook

6. Repeat steps 1 -5 for the remainder of the fabrics listed above.

PART B: Fiber cross sections

1. Obtain 2-3 cotton fibers.

2. Thread them 2-3 cm up the tip of a Pasteur pipet.

3. Pour one or two drops of Norlands 65 into the pipet from the top and allow to run into the tip.

4. When the tip is full cure the Norlands under long wave UV light for 15 minutes.

5. With a single edge razor blade, chip away the glass of the tip and cut a thin section of the Norlands and fiber.

6. Mount in a drop of 1.53 Cargille Refractive Index Oil with a coverslip.

7. Observe under the microscope.

8. Sketch what you see in your lab notebook.

9. Repeat steps 1-8 for the remaining fibers.

PART C: Effect of heating

1. Prepare the apparatus shown on the next page. See Figure 1.

   a. Carefully insert a strip of cotton fabric into the bottom of a test tube.
   b. Insert a strip of both damp red and blue litmus in the top of the tube. Be careful to ensure that the strips do not touch each other.
   c. Cover the test tube with a piece of filter paper.

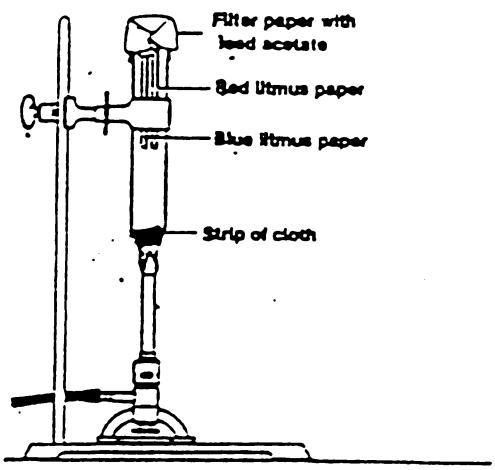

Figure 1: Litmus Set-Up

2. Gently heat the test tube and its contents.

3. Observe the effect of the heating on the litmus papers.

4. Repeat steps 1-4 for the remainder of the fabrics listed on page 1 of this activity.

5. Prepare a chart showing the results of this testing for all of the fabrics.

PART D: Effect of burning on fabric.

1. Pick up a small piece of cotton fabric with a pair of tweezers or forceps.

2. Place in the flame of a bunsen burner.

3. As the fabric burns, evaluate the odor, rate of burning, kind of flame and type of residue.

4. Repeat for the remainder of fabrics listed on the previous page.

5. Prepare a chart comparing the odor, rate of burning, kind of flame and type of residue for all of the fabrics.

SOLUBILITY:

1. Cut a 1 cm x 8 cm piece of cotton fabric into small pieces.

2. Divide the fabric into 4 sections and place into four small test tubes or four wells on a spot plate.

3. Using a Pasteur pipet put twenty drops of 6 M NH_4OH into the first test tube.

4. Using a Pasteur pipet put twenty drops of 6 M HCl into the second test tube.

5. Using a Pasteur pipet put twenty drops of 6 M H_2SO_4 into the third test tube.

6. Using a Pasteur pipet put twenty drops of water into the fourth test tube.

7. Allow to react for at least five minutes.

8. Record the results in your lab notebook.

9. Repeat steps 1-8 for the remaining seven fabrics.

10. Prepare a chart comparing the solubility of the fibers in the four compounds.

DATA: Prepare data tables in your lab notebook as indicated above.

QUESTIONS:

1. Are synthetic or natural fibers easier to identify?

2. Do synthetic and natural fibers appear the same, microscopically?

3. Why was water one of the chemicals used in the solubility lab?

4. What did we use litmus paper in this lab?

REFERENCES:

The Chemistry of Keeping Clean, Teachers Guide. A Module in High School Science. (1989). Michigan State University. Lessons 18-22.

Clifton Meloan, Richard James, Richard Saferstein. (1990). Criminalistics: An Introduction to Forensic Science Lab Manual. New Jersey. Prentice Hall.

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Siegel, Jay. (1993) Forensic Science Laboratory Manual. Michigan State University. East Lansing, Michigan.

Revised by Karen Pawloski

CARPET SAMPLE LAB

The examination of hair and fibers can systematically be completed in the crime lab if the fibers are given to the scientist. Many times however it is the job of the scientist to investigate and find the evidence from vacuum bags and carpet samples. In this lab you will be given a carpet sample which has evidence imbedded in it. Your job is to find the evidence, do a cursory analysis of it and then develop a plan to determine the identity of it. Upon completing the development of the plan you can begin to implement the plan. Your ultimate goal is to identify the type of hair or fiber you have and document all testing procedures and conclusions which led to your final evaluation.

References:

Pawloski, Karen. (1996) Forensic Science in the High School Classroom. Michigan State University, East Lansing.

DETERMINATION OF LIPSTICK DYES USING TLC

The examination of lipstick stains left on clothing, glass, napkins and cigarettes provide valuable clues as to the identity of a suspect. Lipsticks are composed of fats, oils, waxes, colorings, perfumes and flavorings. The color of a lipstick is mainly due to aluminum, calcium or barium dyes in concentrations between 15% and 20%. By first extracting the dyes from the stained material the forensic chemist can then separate the dyes by TLC.

CRIME SCENE

The Michigan State Flag is missing from Mrs. Pawloski's room. It appears that some delinquent has forcefully entered the building last night. A search of the premises revealed a scrawled note across the window in lipstick, "Michigan and mathematics rule". After photographing the note the lipstick will be removed using kimwipes. All three female mathematics teachers are suspects in this horrendous deed. Mrs. Pawloski has confiscated the lipstick from the three suspects. Your job is to analyze the three confiscated lipsticks and the note lipstick and determine which, if any, of the teachers committed this crime.

OBJECTIVES:

The student will separate lipstick dyes using thin layer chromatography.
The student will calculate Rf values from their thin layer chromatography plate.

MATERIALS:

beaker, 250 ml
stirring rod
graduated cylinder, 10 ml
pipet, 1 ml
scissors
test tube rack
4 test tubes, 13 x 100 mm
TLC plates
forceps
micropipettes, 25 ul
methanol
isoamyl alcohol
acetone
distilled water
ammonium hydroxide
lipsticks
evidence on Kimwipes or window
metric ruler
filter paper, 12 cm

PROCEDURE:

1. Obtain a piece of stained Kimwipe. Using scissors cut a 1 x 2 cm section into small pieces.

2. Place the pieces into a small test tube with 5 drops of methanol. (The pieces should be wet and there should be some liquid in the tube. If not add more methanol.)

3. Now make three stained Kimwipes using the three suspected lipsticks.

4. Cut out a 1 x 2 cm section of each marked Kimwipe and place in a small test tube with 5 drops of methanol.

5. Prepare a chromatographic plate by notching the ends as shown in Figure 1. This notching forces the solvent to move through a narrow space, with the result that the dyes will appear as thin bands of color well separated.

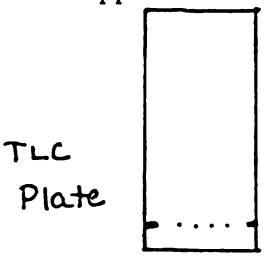

Figure 1

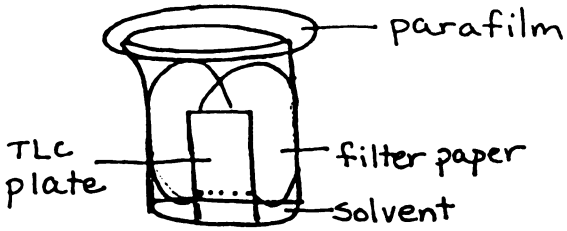

Figure 2

6. Using a micropipette transfer 10 drops of the eluted lipsticks onto the thin plate between the notches. Be sure to allow each drop to dry thoroughly before adding the next drop. Make sure you document which drop is which lipstick as shown in the diagram. You will have a total of 4 separate dots.

*** The lab made be interrupted at this point and finished another day.

7. Prepare the developing solvent. Mix 5 ml of isoamyl alcohol, 5 ml of acetone, 3.2 ml distilled water and 0.1 ml of ammonium hydroxide in the 250 ml beaker. Stir with the stirring rod vigorously, but being careful, to ensure mixing.

8. Place a piece of filter paper into the beaker. The filter paper should be leaning against the wall of the beaker with a small portion submerged in the solvent. The paper will absorb some of the solvent and make the entire atmosphere of the TLC chamber like the solvent. Cover the beaker with parafilm. See Figure 2.

9. Hold the spotted plate next to the chamber to be certain that the colored spot is above the solvent line rather than immersed within.

10. Uncover the chamber and place the TLC plate into it. Replace the parafilm and allow to stand undisturbed until the solvent reaches the end of the plate.

11. Remove the plate and mark the solvent front by making a notch in the TLC plate.

12. Allow the plates to dry and compare the dyes on each.

13. Measure each band from the point of application.

14. Measure the solvent front from the point of application.

15. Calculate the Rf values.

$$Rf = \frac{\text{distance of sample band from application point}}{\text{distance of solvent front from application point}}$$

16. Compare the evidence with the three known chromatograms.

DATA:

Draw and label each of the chromatography plates. Show the distance moved and calculate the Rf values.

CONCLUSIONS:

State who is the likely suspect and why.

QUESTIONS:

1. Why did the lipstick extracts move to different places on the TLC plates?

2. Does this evidence unequivically prove the origin of the note?

3. What other types of crimes might utilize this forensic technique in their solution? Suggest a scenario.

FURTHER INVESTIGATIONS:

Develop a technique for the analysis of other dyes, such as inks or blushes.

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Clifton Meloan, Richard James, Richard Saferstein. (1990). Criminalistics: An Introduction to Forensic Science Lab Manual. New Jersey. Prentice Hall.

Rost, Carolyn. (1990). Lab Manual. Michigan State University. East Lansing, Michigan.

Siegel, Jay. (1993). Forensic Science Lab Manual. Michigan State University. East Lansing, Michigan.

Revised by Karen Pawloski

THE ANALYSIS OF QUESTIONED DOCUMENTS

The analysis of questioned documents (QD) represents one of the few "apprenticeship" fields left in forensic science. By this, it is meant that this area is learned mainly by studying with an experienced QD examiner. There is not much in the way of formal education in this field. Because of the nature of instruction in questioned document analysis, apprenticeships tend to be long and in some cases may be up to 3 years before the trainee is permitted to work on cases solo. By the same token, some of the popularity of this area lies in the opportunities for QD examiners to engage in civil work outside the criminal field, thus enhancing their income.

The great challenge in questioned document analysis lies in the fact that in arriving at a decision concerning a case, the examiner has only his/her own knowledge, skill and experience to draw upon. This contrasts with most other areas of forensic science where there are analytical instrumental techniques which can be used to aid the examiner in reaching conclusions. The complete reliance upon one's own skills puts a great burden upon the examiner.

The purpose of this experiment is to acquaint you with some of the types of QD cases and analyses which the examiner must confront. Since you are not an expert in this field, the exercises are fairly straightforward and basic. They include some analysis of handwriting characteristics, attempts at forgery and analysis of inks.

MATERIALS:

Kit for determining handwriting spacing and slants
Samples of handwriting
TLC apparatus

PROCEDURE:

PART A: Handwriting analysis

During the first class period you were given some dictation which was subsequently collected from you. This is the material which will now be given back to you for analysis. You will be furnished with a "questioned document" and a series of exemplars from different sources. Using the knowledge you have gained from lecture and the slant and spacing analysis kit, you are to decide which, if any, of the knowns came from the same source as the unknown. Some of the characteristics of the handwriting you may want to

look at are slant, intraletter and interword spacings, relative sizes of letters, loop characteristics, proportional size of letters, and unusual characteristics. Remember there is not a set number of characteristics which must be determined in order to arrive at a conclusion. The number is up to the examiner. It is also true, however, that the more characteristics found, the better the result.

PART B: Forgery

Get a member of your group to furnish you with a copy of his/her signature. Practice forging the signature until you are proficient in doing so without looking at the real signature. Then try forging the signature by tracing over it on another sheet of paper. Compare the forgeries to see if you can determine which ones are traced. Turn in your best forgery efforts along with the real signature.

PART C: Thin Layer Chromatography of Inks

See next page.

ANALYSIS OF INKS BY THIN LAYER CHROMATOGRAPHY

This experiment will demonstrate a technique used by forensic scientists. The purpose is to identify colored pigments in pen inks. The need for differentiating inks arises when people prepare fraudulent documents, send ransom notes or threaten others in written form. For example, a student may change an answer on another student's paper. Similarly, a person may attempt to forge an absence note from their parent. Handwriting analysis and the ability to differentiate inks will often permit the forensic scientist to prepare a document and perhaps locate the owner of the pen itself.

Modern day inks are actually composed of mixtures of dyes. These dyes can be separated by TLC. Thin layer chromatography utilizes a thin film of silica gel coated onto a glass of plastic strip. As in paper chromatography, this thin film is called the stationary phase. A mixture of the compound to be separated, in this case inks, is placed in a small spot at one end of the strip, and a liquid, an organic solvent, the mobile phase, is passed over the spot. The chromatographic developing chamber is a small beaker covered with parafilm. As the solvent moves up the strip, it carries the different components within the ink. Because each compound present has a different size, shape and electrical field, each compound will adhere to the stationary phase and dissolve in the mobile phase to a different extent. After a period of time the flow of the mobile phase is stopped, the strip is dried and the pigment distance is measured. The distance the pigment moves relative to the distance the mobile phase moves is called the Rf value.

CRIME SCENE

Mary Williams is receiving threatening notes on her locker. Although she is unsure who is at fault she suspects Terrible Teresa who has been harassing her in Geometry class. Mary has called in the Forensic Crime Team to help solve the crime. How might we go about proving who is responsible for this terrible deed? What suggestions might you propose?

MATERIALS:

3 test tubes, 13 x 100 mm
graduated cylinder, 10 ml
scissors
test tube rack
forceps
beaker, 250 ml
micropipettes, 25 ul
TLC plates
parafilm
methanol
n-butanol
distilled water
isopropanol
pens
written note
metric ruler
filter paper

PROCEDURE:

1. Obtain a piece of the handwritten note. Using scissors cut five words into small pieces.

2. Place the pieces into a small test tube with three drops of methanol. More methanol may need to be added if the paper pieces soak it all up.

3. Make a heavy pen mark on a piece of paper with each of the three pens provided.

4. Cut out each mark and place in separate small test tubes with three drops of methanol.

5. Prepare a chromatographic plate by notching the ends as shown in the diagram. See Figure 1. This notching forces the solvent to move through a narrow space, with the result that the dyes will appear as thin bands of well separated color.

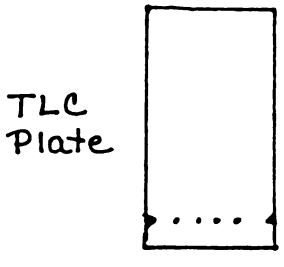

Figure 1

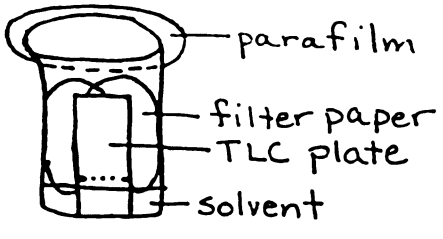

Figure 2

6. Using the micropipette, transfer three drops of the eluted inks onto the thin plates between the notches. Be sure to allow each drop to dry thoroughly before adding the next drop.

*** The lab may be interrupted at this point and held until the next day.

7. Prepare the developing solvent. Mix 10 ml of n-butanol, 5 ml of isopropanol and 5 ml of distilled water in the 250 ml beaker.

8. Place a piece of filter paper against the inside wall of the beaker with it's bottom edge touching the developing solution. Some of the solution will be absorbed by the filter paper creating an atmosphere similar to the solvent in the chamber. Cover the beaker with parafilm.

9. Hold the spotted plate next to the chamber to be certain that the colored spot is above the solvent line rather than immersed within.

10. Place the TLC plate in the developing chamber with the silica gel side facing away from the filter paper. Cover and allow to stand undisturbed until the solvent reaches the top of the plate.

11. Remove them and make a notch to mark the solvent front.

12. Allow the plates to dry and compare the dyes on each.

13. Measure each band from the point of application.

14. Measure the solvent front from the point of application.

15. Calculate the Rf values.

$$Rf = \frac{\text{distance of sample band from application point}}{\text{distance of solvent front from application point}}$$

16. Compare the known ink chromatograms with your handwritten note and determine the origin of the document.

DATA:

Draw and label each of the chromatography plates. Show the distance moved and calculate the Rf values.

CONCLUSIONS:

State which pen was used to write the threatening note.

QUESTIONS:

1. Why did the inks move to different places on the TLC plates?

2. Does this evidence unequivically prove the origin of the note? Why or why not?

REFERENCES:

Rost, Carolyn. (1990) Lab Manual. Michigan State University. East Lansing, Michigan.

Clifton Meloan, Richard James and Richard Saferstein. (1990). Criminalistics: An Introduction to Forensic Science Lab Manual. New Jersey. Prentice Hall.

Siegel, Jay. Forensic Science Lab Manual. Michigan State University, East Lansing, Michigan 1993.

Revised by Karen Pawloski

VISUALIZATION AND EXAMINATION OF FINGERPRINTS

The science of dactyloscopy, or the study of friction ridges is one of the most important methods of personal identification available to the criminal justice system. Friction ridges include finger, palm, foot and lip prints, but the most important is, of course, fingerprints.

There are a number of components to dactyloscopy. They include:

1. Visualizing latent (invisible to the naked eye) prints on a variety of surfaces.
2. Transferring a visualized print to a tape or a card.
3. Obtaining known prints by inking.
4. Comparing inked (known) prints with unknown prints, either latent or patent (visible to the eye without development.)
5. Perform a classification of a set of ten fingerprints using FBI system.

In this experiment, you will learn about several ways of visualizing latent fingerprints. You will also compare an inked print with a visualized latent print. Finally, you will perform an FBI classification on a set of 10 prints.

MATERIALS:

fingerprint powders and brushes
lifting tape and cards
ink pads
magnifying glasses
FBI card
set of unknown fingerprints

PROCEDURE:

PART A:

Have a partner roll your right thumb print onto a white index card. Repeat until you obtain a non - smudged print. Write your name on the card and turn the card in to Mrs. Pawloski.

PART B: Dust and Lift

1. Run your fingers through your hair to increase the amount of oil on your fingers.

2. Deposit some fingerprints on surfaces such as a beaker, glass plate or a piece of paper.

3. Using a powder that contrasts in color with the object that you are dusting, lightly dust the print.

4. Examine the dusted print with a magnifier to see if you can clearly see and count the ridges. This is the basis for comparison of fingerprints.

5. Using lifting tape, lift one of your best dusted prints. Start by putting the tape down on the left side of the print. Then, using a finger as a roller, push the tape down across the print in one motion.

6. Lift the tape and transfer it to a contrasting card (black or white side).

7. Tape the print to the card.

PART C: Silver nitrate visualization.

1. Spray or brush an object with a silver nitrate solution (5 g $AgNO_3$ in 100 ml of distilled water). Since fingerprints contain some sodium chloride, insoluble silver chloride will form and be deposited along the friction ridges.

2. Wash the object in water (gently) to remove the excess silver nitrate and put in a drawer (complete darkness) to dry.

3. After the object dries, put it in the light. Light will catalyze the decomposition of silver chloride to from free silver which is black.

4. After the print outlines are black, dip the object into photographic hypo clearing agent to remove the excess silver chloride and fix the silver image. Failure to fix the silver will result in its turning the print completely black.

PART D: Fingerprint comparison

Using the same finger that made your best dusted and lifted print, make an inked impression on a piece of paper. Find at least 12 points of identification on the two prints and label them with lines and numbers.

PART E: FBI classification

1. Using the FBI fingerprint card given you, make an inked set of prints for you and your partner.

2. Classify each of your fingerprints as the fingerprint type (ulnar loop, tented arch, plain whorl, etc.).

3. Using the information from step 2 determine your FBI primary classification.

PART F: Unknown determination

1. Obtain a sheet of fingerprint unknowns from Mrs. Pawloski.

2. Determine which print is your own.

3. Find at least 12 points of comparison between the selected unknown print and your inked print. Label them with lines and numbers.

DATA:

Submit the following to Mrs. Pawloski:

1. Example of dust and lift - matched to 12 points with a known inked print.

2. FBI card with fingerprint types and FBI primary classification.

3. Matched unknown print.

QUESTIONS:

1. Why is doing an FBI primary classification on a set of prints useful to a fingerprint examiner?

2. What works better when rolling a set of prints, a little or a lot of ink? Why?

3. Look at the set of unknown prints from our class, what print type is most common? What is least common?

4. Write the chemical equation for the reaction of silver nitrate with the sodium chloride on our skin.

REFERENCES:

Siegel, Jay (1993). Forensic Science Lab Manual. Michigan State University. East Lansing, Michigan.

Revised by Karen Pawloski

SHOEPRINTS

Among the types of evidence which is individualized to a particular source are shoeprints and tire treads. The print or tread must be left in a material which is capable of "holding" it after deposition. Certainly the most common among these substances is soil. In a typical case, a shoeprint from a shoe which has some characteristic tread pattern is deposited in soil. A cast of the print in the soil is made and then compared to the pattern in the shoe. In this case, older worn shoes are much easier to individualize than are brand new shoes because the former have undergone random wear and tear which is not expected to be reproduced exactly on any other shoe sole. If enough of these characteristics can be captured by the cast, then the shoe can be individualized to the cast and hence, the imprint.

MATERIALS:

quick dry plaster
bucket, water, etc.
twigs or metal screen
camera

PROCEDURE:

1. Depending on the weather, you may be able to make a shoeprint outside in a patch of dirt, otherwise use the plastic tub of dirt in the laboratory. Using a shoe with a tread pattern, preferably one that shows some wear and tear, make an impression by walking through the dirt patch or tub of soil.

2. When you are ready to take the impression, make enough plaster to cover the entire impression and leave a six inch border all the way around it. Use only enough water with the plaster to make a thick paste. Pour in the plaster to a depth of about 1 inch and then lay several twigs or some metal screen on top of the cast. It is a good idea to have the twigs extend out from the border so that they can be used to grasp the cast while carrying it. Then pour another inch or so on top of the twigs or screen to complete the cast.

3. When the plaster sets (about 30 min), remove the cast and put it in the lab, impression side up and leave alone for at least 24 hours.

4. Gently scrape off all dirt and debris from the impression. You may use water to wash off the residue but scrape off as much as possible into a trash receptacle first so that you avoid putting too much dirt down the sink drain.

5. Using printers ink, ink the bottom of the shoe and make an impression on a piece of blank white paper. Then compare the inked impression and the cast by finding and marking unique points which help to individualize the shoeprint and the shoe.

6. Photograph the inked impression, the shoe and the cast. The pictures you take which are labeled with the points of ID, will be your evidence in court of your match. Find as many points of identification as you can.

DATA:

Turn in your inked impression and your plaster cast.
Turn in your points of comparison.

QUESTIONS:

1. Besides plaster, what other materials could be used to lift a shoeprint impression? What are their relative advantages and disadvantages?

2. Describe a suitable method for making a shoeprint cast from an impression in the snow.

REFERENCES:

Siegel, Jay. (1993). Forensic Science Lab Manual. Michigan State University. East Lansing, Michigan.

Revised by Karen Pawloski

URINE ANALYSIS USING PAPER CHROMATOGRAPHY

Chromatography techniques are methods used to physically separate mixtures of gases, liquids, or dissolved substances. Separation is determined by the molecular size and/or charge of the individual components in the sample mixture.

All forms of chromatography have two things in common: a stationary (absorbent) phase, and a mobile phase. The stationary phase is the material on or in, which the separation takes place. The paper is the stationary phase in paper chromatography. The solvent is the mobile phase that dissolves the sample forming a solvent-sample mixture and transports the sample over the stationary phase to facilitate the separation of the components.

The sample is first placed on the paper at a fixed point. The tip of the paper is then placed in the solvent, taking care not to immerse the sample itself. The solvent, "carrying" the sample with it, begins to travel up the paper. As it does, the individual sample components may travel at different rates and separate. The components that are held less "tightly" by the stationary phase will travel the fastest.

The distance each component travels from the origin is called the solute front, and the distance the solvent travels is called the solvent front. The ratio of these measured values is called the Rf (rate of flow) value. These ratios are determined by the formula:

$$Rf = \frac{\text{distance solute traveled}}{\text{distance solvent traveled}}$$

In paper chromatography the rate at which individual components move is related to how easily each component is dissolved by the solvent (mobile phase) being used. Whichever component is most easily, and most completely, dissolved by the solvent is the component that will move the farthest in the time allowed. Some other factors that may influence how fast and far a component moves are: molecular size (with smaller molecules moving faster), electrical charge, and how great an "attraction" (usually determined by charge) the component has for the stationary phase. In paper chromatography solubility is the most important factor and the most soluble component will have the highest Rf value.

The Rf value for each component is unique and can be used to identify individual components when compared to known standards.

MATERIALS:

chromatography paper	food coloring:
two sheets plain white paper	blue
eye protection	red
1000 ml beaker	yellow
scissors	green
pencil (not pen)	four capillary tubes
distilled water	ruler
tape	paper toweling

the food color simulates urine samples

PROCEDURE:

1. Add 100 ml of water to the beaker.

2. Cut four pieces of chromatography paper 17.5 cm in length (width should be 2.54 cm).

3. Cut one end of each piece diagonally twice to form a pointed end similar to a stake.

4. Use a pencil to draw a line perpendicular to the length of the chromatography paper; 2.54 cm from the bottom of the tip as shown in Figure 1. This will be the sample origin.

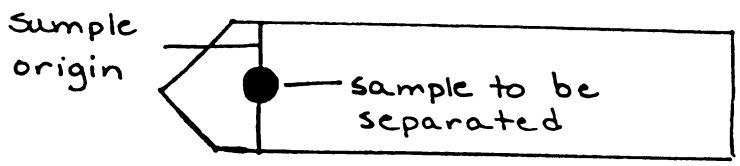

Figure 1

5. Mark a small dot at the center of this origin line with pencil.

6. Label the top of each of the four pieces of chromatography paper with one of the appropriate food colors that are to be separated.

7. Use a capillary tube (be sure not to mix the tubes) to transfer a small amount of the food coloring to the dot. Let the sample dry for 30 seconds and repeat this transfer two more times.

8. Allow the sample to dry for one minute after the final transfer.

9. Repeat steps 7 and 8 for the remaining three colors - each on a separate strip of paper.

10. Gently slide the pointed end of each strip down the interior of the 1000 ml beaker until the tip comes in contact with the water. Make sure the sample doesn't come in contact with the water.

11. Bend the upper end of the paper over the rim of the beaker and tape if necessary.

12. Allow approximately 15 - 20 minutes for the fool colors to separate. Watch the progression of the solvent front, and let it move to within 1 or 2 cm of the beaker rim.

13. Remove the chromatography paper from the beaker after the samples have separated and place it on paper toweling to dry.

14. Use a pencil to draw a perpendicular line representing the farthest distance traveled by the solvent. This is your solvent front.

15. Tape two paper strips on a 8 1/2 x 11 inch piece of white paper. Place the tape above the solvent front and below the origin. Your partner should do the same for the other two strips.

16. When the samples have dried, use a pencil to draw perpendicular lines through the middle of the separated components. This is the solute front.

17. Measure the distance from the origin to the solute front for each component. Measure the distance from the origin to the solvent front for each strip.

18. Wash hands thoroughly with soap and water.

DATA:

Draw a diagram of each of the four strips showing their band patterns.

QUESTIONS:

1. Calculate the Rf values for all separated components.
2. Why is pencil used instead of pen?
3. How many components does each sample have?

REFERENCES:

Schlitz, Gary. (1994). Forensic Laboratory Science and Detective Writing. Illinois. Flinn Scientific, Inc.

Revised by Karen Pawloski

OUCHTERLONY DIFFUSION

Blood is often left at the scene of a crime. The criminalist must be prepared to answer the following questions when examining blood:

1. Is it blood?
2. Is it human blood?
3. If it is human can we characterize the blood type?

The simulated blood samples we are using today have been characterized as blood. This was accomplished by treating the stain area with phenophthalein. When blood stains, phenolphthalein and hydrogen peroxide are mixed, the blood's hemoglobin will form a deep pink color.

Once the stain has been characterized as blood the researcher must determine if the stain is human. Today we will use the ouchterlony diffusion method to simulate this type of identification. This test is based on the fact that when animals (usually rabbits) are injected with human blood, antibodies are formed that react with the invading human blood. The investigator can recover these antibodies from the rabbit by bleeding the animal and isolating the sera. This rabbit serum will contain antibodies that specifically bind to human antigens. For this reason, the serum is known as human antiserum. In the same manner, by injecting rabbits with other known animals blood other antisera can be produced.

Today we will utilize the process of gel diffusion, taking advantage of the fact that antigens and antibodies will move toward each other on a gel plate. Here, the extracted bloodstain and the human antiserum are placed in separate wells opposite each other on the gel. If the blood is of human origin, a line of precipitation will form where the antigens and antibodies meet. This test is extremely sensitive and requires only a small thread of blood soaked material. Human bloodstains dried as long as 10 - 15 years still provide positive results. Extracts from mummies 4000 to 5000 years old have given positive reactions.

MATERIALS:

small petri dishes - 35 mm 1/student pair
soda straws
wax pencils
agarose
NaCl
human antibody (0.5 M Na_2SO_4)
human antigen (0.5 M $BaCl_2$)
simulated blood soaked cloth
same cloth, unstained
pasteur pipets

PROCEDURE:

PART A: Preparation of the ouchterlony media.

1. Dissolve 1.76 grams of NaCl in 200 ml of distilled water.

2. Dissolve 2.4 grams of agarose in the saline solution. It will be necessary to heat the agarose solution to cause the agarose to dissolve.

3. Pour enough agarose solution into each petri dish to cover the bottom of the plate to a depth of about 5 mm.

4. Allow the plates to cool overnight.

PART B: Preparation of the ouchterlony wells.

1. Using the template in Figure 1, as a guide, cut small well with a soda straw in the agarose.

2. Carefully remove the wells with a Pasteur pipet.

3. Mark 12:00 o'clock with a wax pencil on the dish containing the wells and familiarize yourself with the standard numbering system provided in Figure 1. Notice it is not necessary to number the wells on the dish since the mark can be used as a reference point.

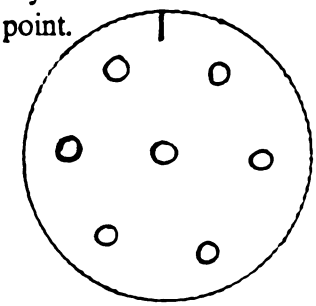

Figure1

PART C: Filling the wells.

1. Fill the center well with three drops of human antibody.

2. Place a 1 cm square of stained cloth in a test tube. Add 5 drops of water and stir thoroughly.

3. Place a 1 cm square of unstained cloth in a test tube. Add 5 drops of water and stir thoroughly.

4. Fill well #1 with 3 drops of the questioned stain antigen, well #3 with 3 drops of the known human antigen and well #5 with 3 drops of the unstained cloth eluate.

5. After you have filled the wells allow the plate to develop for 30 minutes.

PART D: Recording results.

1. Look through the bottom of the plate while holding it up to a bright light.

2. Make a drawing of the plate and the results that you observe. If the reaction is positive, a precipitin line will have formed between the center well and the adjacent well. Make a "+" (positive reaction) or "-" (negative reaction) in the space between the wells.

DATA:

Make a drawing of the plate showing the results.

QUESTIONS:

1. Why is it necessary to eluate the unstained cloth?

2. What further tests would the criminalist perform with this blood sample?

3. Explain the antigen-antibody response.

TEACHER'S GUIDE:

To prepare the blood soaked cloth, soak a piece of cloth in a 1 M barium chloride solution mixed with red food coloring.

REFERENCES:

Clifton Meloan, Richard James, Richard Saferstein. (1990). Criminalistics: an Introduction to Forensic Science Lab Manual. New Jersey. Prentice Hall.

Rost, Carolyn. (1990). Lab Manual. Michigan State University. East Lansing, Michigan.

Washburn, Sherwood 1.; The evolution of man. Sci. Amer. September: 194 - 208: 1978.

Revised by Karen Pawloski

DETERMINATION OF THE PEAK ABSORBANCE OF A SUBSTANCE USING VISIBLE SPECTROPHOTOMETRY.

Visible spectrophotometry is the measurement of the amount of light absorbed by a colored solution, the amount absorbed being proportional to the concentration of the compounds in the solution. Each colored compound has a select few wavelengths that it will absorb in preference to other wavelengths. Therefore, by carefully selecting these wavelengths, it is often possible to measure the amount of one colored compound in the presence of others.

In this lab, you will determine the wavelength that a colored solution will maximally absorb. To do this you will make a series of dilutions of methylene blue dye and test each dilution at a range of wavelengths. The absorbance values will then be plotted on a graph of absorbance versus wavelength and a peak wavelength will be established.

MATERIALS:

7 - Spec 20 cuvettes
distilled water
.025% methylene blue stain
(prepare by serial dilution of .1% stain)
Spectrophotometer

PROCEDURE:

PREPARATION:

1. Turn on Spectrophotometer to allow time to warm up.

2. Serially dilute a 0.025% solution of methylene blue stain into 6 cuvettes as follows:

    a. Place 4 ml of .025% methylene blue in the first cuvette.
    b. Transfer 2 ml of the .025% methylene blue solution from the first cuvette to the second cuvette.
    c. Add 2 ml of distilled water to the second cuvette.
    d. Mix the solution in the second cuvette thoroughly.
    e. Repeat this process from cuvette 2 to 3 and so on until all six cuvettes have solution in them.
    f. Determine the percent of methylene blue in each tube.

3. Prepare a blank by placing 4 ml of distilled water in the seventh cuvette.

TESTING:

1. Set the spec 20 to a wavelength of 340 nm.

2. With the sample chamber empty and the cover closed, use the power switch knob on the left to set the meter needle to infinity absorbance. (When the chamber is empty, a shutter blocks all light from the phototube.)

3. Wipe the blank free of fingerprints and moisture with a Kimwipe. Insert the tube into the sample holder, close the cover and set the meter to zero absorbance using the right knob.

4. Repeat steps 2 and 3 until the readings are repeatedly correct. (Without the blank the instrument should automatically go back to infinity absorbance and with the blank to zero absorbance.)

5. Wipe cuvette 1 free of fingerprints and insert into the sample holder.

6. Read the absorbance at 340 nm and document.

7. Change the wavelength by 20 to 360 nm.

8. Read the absorbance and document.

9. Repeat steps 7 - 8 (i.e. change by increasing 20) until a wavelength of 700 nm is reached.

10. Remove cuvette 1 from the sample holder and repeat steps 2 - 4.

11. Repeat steps 5 - 10 for the remaining five cuvettes.

DATA:

1. Prepare a data table of wavelength and absorbance for all six cuvettes.

2. Plot the absorbance (y-axis) vs. wavelength (x-axis) on a graph.
   Data for all six tubes can be plotted on one sheet of graph paper.
   Different colored pencils can be used to represent each tube.
   After plotting the data, connect the dots using straight lines.

CONCLUSION:

Look at your graph of the data and determine the maximum absorbance for each tube. You should see a common peak between all six of the tubes.

QUESTIONS:

1. What was your peak absorbance value?

2. Why did you determine this?

3. Suggest sources of error in this experiment.

REFERENCES:

Clifton Meloan, Richard James, Richard Saferstein. (1990). Criminalistics: An Introduction to Forensic Science Lab Manual. New Jersey. Prentice Hall.

Pawloski, Karen. (1996). Forensic Science in the High School Classroom. Michigan State University. East Lansing, Michigan.

IDENTIFICATION OF SALICYLATES IN BLOOD BY SPECTROSCOPY

Visible spectroscopy allows the researcher to measure the amount of light absorbed by a colored solution, the amount absorbed being proportional to the concentration of the compounds in the solution. Each colored compound has a select few wavelengths that it will absorb in preference to other wavelengths. Therefore by carefully selecting the wavelengths desired, it is often possible to measure the amount of one compound in the presence of several others.

Salicylates react with ferric salts to produce a violet color, which is proportional to the concentration of the salicylate. For the detection of the salicylate we will measure light absorption at 540 nm. Because of the simplicity of this salicylate procedure, the assumption of salicylate poisoning produces a very strong and easily seen violet color with the ferric salt reagent.

Our job today involves developing the purple salicylate colors for several known concentrations, preparing a calibration curve and then determining the concentration of a salicylate in an unknown. In the second part of the experiment we will determine the level of salicylate concentration in a blood serum sample.

TURN ON THE SPEC 20 SO THAT IT HAS AMPLE TIME TO WARM UP!

MATERIALS:

Spec 20
8 cuvettes
pipettes, 1.0 ml and 2.0 ml
test tube rack
ferric nitrate solution, 1%
nitric acid solution, 0.07M
nitric acid solution, 0.039 M
salicylic acid solution (10mg/dL)

PROCEDURE:

PART 1: Basic spectrophotometry and forming a calibration curve.

1. Place six spectrophotometer cuvettes in a small test tube rack. Label.

2. Pipet the amounts of materials into each cuvette as shown in Table 1.1. Calculate the amount of salicylic acid sample and record the results. Hint: Cuvette 2 has a salicylate concentration of 0.5 mg/dL.

3. Set the wavelength dial to 540 nm.

4. With the sample compartment empty, adjust the meter to read 0% T. This is accomplished by rotating the on-off switch.

TABLE 1: Preparation of Standards

	100 mg/dL Standard	distilled water	dilute ferric nitrate	0.039M nitric acid
Cuvette 1	0.0	2.0	0.0	2.0
Cuvette 2	0.2	1.8	2.0	0.0
Cuvette 3	0.6	1.4	2.0	0.0
Cuvette 4	1.0	1.0	2.0	0.0
Cuvette 5	1.4	0.6	2.0	0.0
Cuvette 6	2.0	0.0	2.0	0.0

YOU ARE NOW READY TO TAKE YOUR READINGS

5. Place cuvette 1, the blank in the sample compartment and set the meter at 100% T (zero absorbance) by adjusting the % T knob.

6. Insert each of the known salicylic acid standards in the sample compartment and obtain an absorbance reading for each sample.

7. Plot absorbance (A) on the vertical axis of a sheet of graph paper and the concentration (mg/dL) along the horizontal axis for cuvettes 1 - 6. Draw the best line through these points. This is called a calibration curve.

8. Now pipet 2 mL of the unknown solution into a cuvette, and mix with 2 mL of dilute ferric nitrate. Determine it's absorbance.

9. From the calibration curve and the absorbance reading you obtained for the unknown, determine the concentration of the salicylate in the unknown.

PART 2: Determination of Salicylates in Blood Serum

CRIME SCENE:

A police officer observes a car moving quite erratically down the highway, and, in fact, it even crosses over the centerline, forcing another vehicle off of the road. The officer stops the car, and the driver states that she had a severe headache and had taken some aspirin. She had trouble removing the safety cap on the bottle while driving, and this had caused the erratic driving. No aspirin container is found, the driver saying that she threw it away. The driver denies that she is under the influence of alcohol or other drugs. She is taken to the hospital, where she voluntarily provides blood for an alcohol and drug test. You now have a small sample of the blood serum and are to test it for the presence and/ or concentration of aspirin.

PROCEDURE:

1. Label two small cuvettes each for both the unknown sample and the 10mg/dL standard.

2. The unknown sample cuvettes will be labeled "test" and "blank," and the standard sample cuvettes will be labeled "test" and "blank."

3. Into each cuvette, pipet 2.0 mL of distilled water.

4. Into the cuvettes for the unknown sample, pipet 0.2 mL of serum.

5. Into the cuvettes for the standard, pipet 0.2 mL of the 10 mg/dL standard.

6. Into the blank tubes, pipet 2.0 mL of 0.039 M nitric acid.

7. Into the test tubes, pipet 2.0 mL of dilute ferric nitrate solution.

8. Shake all tubes to mix the contents.

9. Let all tubes stand for 5 minutes.

10. Determine the absorbance of each tube using the spectrophotometer. Record the absorbance on your lab sheet.

DATA SHEET

PART A: Basic spectrophotometry

	Concentration of salicylate (mg/dL)	absorbance
Cuvette 1		
Cuvette 2		
Cuvette 3		
Cuvette 4		
Cuvette 5		
Cuvette 6		

Absorbance of unknown_____

Concentration of unknown obtained from graph:_____ mg/dL

Concentration of unknown (obtained from instructor)_____ mg/dL

% error _____

PART B: Salicylates in blood serum

Standard 10mg/dL	Absorbance
Standard blank	
Standard 10 mg/dL	
Unknown blank	
Unknown	

CALCULATIONS:

Use the relationship below to determine the concentration of salicylate in the unknown sample.

$$\text{mg/dL} = \frac{\text{absorbance of unknown minus absorbance of unknown blank}}{\text{absorbance of standard minus absorbance of standard blank}} \times 10$$

QUESTIONS:

1. Do you suppose that there would normally be salicylate in human blood?

2. Give an example of where this type of procedure might be used elsewhere.

TEACHER'S GUIDE:

Materials:

Ferric Nitrate = 1%

Nitric Acid = 0.039 M
5 parts of 0.07 M and 4 parts of distilled water

Nitric Acid = 0.070 M
.29 ml of concentrated acid to 100 ml of water

Salicylic Acid solution:

Dissolve one aspirin per 500 ml of water. Add .15 g of NaOH pellet. Now take 80 ml of the above and bring to 500 ml. The concentration of the solution is 10 mg/dL. Test the solution before using with students.

OR

Salicylic stock solution is preferred. Dissolve 0.8 g of sodium salicylate in a one liter flask. Add a few drops of chloroform as a preservative, then store in the refrigerator. This solution is equivalent to 50 mg/dL. Dilute with distilled water for clinical use to 10 mg/dL.

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Revised by Karen Pawloski

THE ALCOHOL BREATHALYZER TEST: A LABORATORY SIMULATION

Everyone is familiar with the sight of a motorist pulled to the side of the road by a police officer. Various physical tests, walking a straight line, touching the tip of the nose with a forefinger may lead to a suspicion that the driver is under the influence of alcohol. These dexterity tests alone will not serve as proof in a court of law to establish drunkenness. The legal proof depends on the results of a breathalyzer test administered to the driver at the scene. Legally, a person is under the influence when his blood alcohol concentration has reached a concentration of 0.1 g/ 100mL of blood. The table below summarizes the usual symptoms associated with various concentrations.

Alcohol concentrations	Effects
0.05 %	None
0.05 - 0.10 %	Impaired stereoscopic and night vision
0.10 - 0.15%	Euphoria, lack of inhibition, slow reaction time
0.15 - 0.20%	Moderate poisoning, very slow reaction time, loss of inhibition, disturbances in balance and coordination
0.20 - 0.25%	Severe poisoning, poor balance and coordination, slow thinking, confusion
0.35 - 0.40%	Deep coma, possible death

The chemistry involved in the breathalyzer test is based on the reduction of potassium dichromate by ethanol in an acidic medium to produce chromium trisulfate and acetic acid. The pertinent balanced equation follows:

$$3C_2H_5OH + 2K_2Cr_2O_7 + 8H_2SO_4 \longrightarrow 3CH_3COOH + 2Cr_2(SO_4)_3 + 2K_2SO_4 + 11H_2O$$

(yellow) (green)

In an actual breathalyzer test, the subject must expel 65 mL of ethanol saturated air into a balloon test chamber containing the dichromate solution. Because of the difficulty working with gases, we will simulate this using an aqueous solution.

Oxidation of an alcohol to produce a carboxylic acid is a common general reaction studied in most elementary organic chemistry classes. In this laboratory we will use a

spectrophotometer to quantify the amount of potassium dichromate which combines with ethanol to form chromium trisulfate. We will actually be measuring the absence of yellow from the test tubes.

MATERIALS:

Breathalyzer solution	13 x 100 mm test tubes
0.40% ethanol stock solution	graduated cylinder, 10 mL
0.20% ethanol standard	parafilm
0.10% ethanol standard	Pasteur pipets
0.05% ethanol standard	distilled water
unknown ethanol standard	spectrophotometer

PROCEDURE:

PART A: Oxidation of ethanol with potassium dichromate

1. Prepare 6 test tubes as follows:
    a. Place 2 mL of 0.40% ethanol stock solution into test tube #1. This is your 0.40% standard.

    b. Place 2 mL of 0.20% ethanol standard solution into test tube #2.

    c. Place 2 mL of 0.10% ethanol standard solution into test tube #3.

    d. Place 2 mL of 0.05% ethanol standard solution into test tube #4.

    e. Place 2 mL of distilled water into test tube #5. This is your 0.00% standard.

    f. Place 2 mL of your unknown into test tube #6.

    ** Don't forget to properly label your test tubes.**

2. Place 2 mL of breathalyzer solution into each tube. Cover each tube with parafilm and invert to mix.

3. Allow the reaction mixture to sit for 15 minutes.

PART B: Reading the absorbance of the samples.

    NOTE: The spectrophotometer will need to warm up 15 minutes prior to using it.

1. Set the spectrophotometer wavelength to 410 nm.

2. With the sample chamber empty and the cover closed, use the power switch knob on the left to set the meter needle to infinity absorbance. (When the chamber is empty, a shutter blocks all light from the phototube.)

3. Prepare a blank tube by placing 4 mL of distilled water into a test tube. Wipe the blank free of fingerprints and moisture with a Kimwipe. Insert the tube into the sample holder, close the cover and set the meter to zero absorbance using the right knob.

4. Wipe each sample tube with a Kimwipe, insert it into the sample chamber and read its absorbance. Record your data in the appropriate space in the accompanying data table.

5. Plot absorbance versus concentration on a graph. Draw the best straight line through your data points. Remember that you may not connect the dots.

6. Find the point on the line which corresponds to the absorbance of your unknown sample. Read its concentration and report it in your lab write-up.

DATA:

SAMPLE CONCENTRATION	ABSORBANCE
0.00%	
0.05%	
0.10%	
0.20%	
0.40%	
unknown	

QUESTIONS:

1. What sources of error might be present in this procedure? What steps could you take to correct them?

2. Absorbance of a substance is a linear function based on concentration. The formula for absorbance is called Beer's law (A = E x b x c) where E is equal to a constant extinction coefficient, b is equal to path length of light through the cell and c is equal to molar concentration. Calculate the extinction coefficient for this reaction.

TEACHER'S GUIDE

MATERIAL PREPARATION:

Breathalyzer solution:

You need 0.123% potassium dichromate in 50% sulfuric acid. To prepare measure .123 g of potassium dichromate and dissolve it in 100 mL of 50% sulfuric acid.

OR

Measure 1.23 g of potassium dichromate and dissolve it in 100 mL of 50% sulfuric acid. Take 10 mL of this solution and dilute with 90 mL of 50% sulfuric acid.

Ethanol standards:

To prepare 0.04% ethanol stock solution dilute 0.50 mL of absolute ethanol (95% will work) diluted to 100 mL with distilled water.

To make the remainder of the standards, do 1 : 1 serial dilutions of the ethanol and water.

Unknown ethanol samples:

Unknown ethanol samples can be made up to any concentration for use. I would recommend keeping the level to under 0.40%, since concentrations above that are possibly lethal. The calculation for determining concentration is:

$$\frac{\text{mL of solution desired}}{1} \times \frac{\text{desired \% in g}}{100\text{ g solution}} \times \frac{1.0\text{ g solution}}{1.0\text{ ml solution}} \times \frac{1\text{ ml ethanol}}{0.7907\text{ g}}$$

HAZARDS:

Potassium dichromate is a strong oxidizing agent. Concentrated sulfuric acid used at 50% strength to dilute the dichromate is very corrosive to the skin. In addition, the breathalyzer solution may stain skin or clothing. The process of dissolving the dichromate in acid is

exothermic. The flask will get very hot. Perform the dilution under the fume hood in an ice bath.

DISPOSAL OF WASTES:

Pour all waste solutions into a large beaker. Add a two fold excess of 50% sodium thiosulfate and adjust the resultant pH to about 2 or 3 using 3M sulfuric acid. Allow the mixture to stand for about 1 hour. Neutralize the solution using sulfuric acid or sodium hydroxide to a pH of 7. Flush the solution down the drain with an excess of water. Dry any solid residue and dispose of it in an approved manner.

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Revised by Karen Pawloski

AGAROSE GEL ELECTROPHORESIS USING DYES

The agarose gel is a semi-solid solution that resembles "Jello" and is allowed to solidify in the gel tray. In short, the students will be loading 7 dyes into small holes made in the gel. These holes will be referred to as "wells". After the dyes are loaded into the wells a power supply will be connected to opposite ends of the gel. The dyes will immediately begin migrating through the gel towards a specific electrode.

It is important that students understand the principles involved with the gel electrophoresis. To put simply, the larger the molecule traveling through the gel, the slower it will travel. This is exactly how DNA fingerprinting is done. If a gene fragment is smaller than another fragment, it will run faster and farther through the gel. In this manner DNA fragments can be separated and compared.

Using the dyes in place of the gene fragments is an excellent way to show the same principles. The movement of the dyes in this electric field can be used to illustrate the following:

(1) The migration direction of the dyes can be used to show its overall negative or positive charge. For example, if the dye migrates towards the positive electrode (called the anode) the overall net charge of the dye is negative.

(2) To illustrate how some dyes' molecular make-up are somewhat larger than others by moving slower through the gel.

(3) To show that some dyes can be used as standards to calibrate an agarose gel. Dyes such as xylene cyanol, bromophenol blue and orange-G are accurate size markers for small DNA fragments. Remember DNA fragments have an x-amount of base pairs. The "Base Pair Equivalents" for these dyes are: orange-G, 70 base pairs; bromophenol blue, 250 base pairs; and xylene cyanol, 2800 base pairs.

MATERIALS:

Each team of three will need:

electrophoresis chamber
15-50 power supply
600 mL beaker
100 mL graduated cylinder
325 mL of Tris Borate EDTA buffer (TBE)
300 mL Ehrlenmeyer flask
0.5 g agarose (makes 2 gels)
heat source (bunsen burner works fine)
micropipette syringe

dye samples:
#1 orange - G
#2 pyronin - Y
#3 phenol red
#4 bromophenol blue
#5 crystal violet
#6 xylene cyanol
#7 unknown dye mixture

PROCEDURE:

PART A: Agarose preparation (makes 2 gels)

1. Tape the ends of the gel tray as described by the teacher.

2. Next tape the comb into place as described by the teacher. Be sure to place the comb about 1/3 from one end.

3. Place 0.5 g agarose in the 300 mL Ehrlenmeyer flask and then add 55 mL of TBE buffer.

4. Place the flask on the heat source and bring the solution to a mild boil until all of the agarose has dissolved.

5. Allow the solution to cool for about 3 - 5 minutes.

6. Using a pasteur pipet, lay a thin bead of agarose where the tape meets the tray and allow to cool and solidify.

7. Next pour the agarose into the tray to about 1 - 2 mm from the top and allow to cool until the gel has solidified.

PART B: Loading the electrophoresis unit:

1. Carefully remove the comb from the gel. This will leave small holes in the gel. These small holes are called "wells".

2. Remove the rest of the tape on the ends of the tray.

3. Place the gel tray in the electrophoresis box.

4. Fill the box just to the very top edge of the gel with TBE buffer.

5. Very carefully add the dyes using the micropipette to the wells. Approximately 5 microliters of each will be sufficient. Be sure to draw and label a diagram of the order of the dyes. The order from the materials list works great.

NOTE: The dyes are more dense than the buffer, so therefore they sink to the bottom of the wells. Be sure not to overflow the wells.

PART C: Running the electrophoresis.

1. Carefully place the top on the box.

2. Connect the power supply to the electrophoresis top. Be sure not to turn on the power yet until all leads are connected. Attach the positive electrode (red) to the side of the tray that has 2/3 of the gel exposed. The negative lead should be attached to the side of the tray that has only 1/3 of the gel. Turn on the power supply and adjust it to about 30 volts.

3. Closely watch for the initial migration of the dyes to be sure all of the connections are satisfactory. The colored dyes should move out of the well and into the agarose gel.

4. Immediate observations can be made about the net overall charge of the dyes but the power should be left on until the dye mixture (#7) separates into 3 distinct colors.

DATA AND ANALYSIS:

Record your observations in the chart below.

DYE	SPEED OF MOVEMENT	NET OVERALL CHANGE
#1 orange-g		
#2 bromophenol blue		
#3 pyronin-Y		
#4 crystal violet		
#5 phenol red		
#6 xylene cyanol		
#7 dye mixture		

DISCUSSION AND QUESTIONS:

1. What dyes were positive and which electrode did they migrate towards?

2. Compare the size of gene fragments in terms of the speed that they might travel through the agarose gel.

3. What were the three unknown dyes in the dye mixture?

4. Of the three dyes that were used as standards, which one of them is probably the biggest molecule and how can you tell by looking at your gel?

5. Approximately how many base-pair equivalents would you guess phenol red to be? (Hint: compare it to your standards).

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Revised by Karen Pawloski

DNA ELECTROPHORESIS

Electrophoresis is a separation technique based on the observation that a molecule will migrate in an electrical field. In the case of DNA, a negatively charged molecule, the migration will be towards a positive pole. If this process can be retarded somewhat by forcing the molecules to move through a dense medium differences in charge and size of the molecules can be seen. Gel electrophoresis, used in this lab exercise, provides the dense material needed.

In order for differences in sizes of DNA to be seen in electrophoresis, the DNA molecule needs to be cut in some way. Restriction enzymes are used to make these cuts. Restriction enzymes are specific for certain sequences of base pairs of DNA. They will only cut if the exact sequence is present. This produces the wide variance in the DNA electrophoresis of humans.

In this procedure, we will be casting agarose gels, loading the gels with DNA which has been previously cut with restriction enzymes, placing the gel into an electrical field, electrophoresing it, and finally staining and destaining the gel. Though this appears to be very complex, it is actually a very simple technique which has become the basis for DNA profiling.

MATERIALS:

electrophoresis chamber
TBE buffer
restriction cut lambda DNA
1% agarose gel
distilled water
staining trays
power supply
micropipettes, 15 ul
loading dye
0.025% methylene blue stain
ruler

PROCEDURE:

PART A: Buffer preparation

1. Prepare the buffer according to the following:

    Add: 12.1 g Tris Base
    0.68 g Boric Acid
    0.29 g EDTA
    To: 1 liter of distilled water

2. Mix thoroughly.

PART B: Agarose gel preparation

1. Measure 0.5 g of agarose.

2. Add this to 50 mL of TBE buffer prepared in Part A.

3. Heat the agarose, mixing frequently, until the agarose is completely dissolved.

4. Let the solution cool slightly.

5. While the solution is cooling, tape the ends of the gel tray.

6. Tape the comb into place. This will form the wells for placement of DNA samples.

7. Pour the agarose into the prepared gel tray and allow the gel to cool until solidified. (The gel tray may be wrapped in Saran wrap and refrigerated overnight, at this point.)

8. Remove the comb, carefully, and remove the tape at the ends of the gel tray.

9. Place the gel and the gel tray into the electrophoresis chamber.

10. Pour the TBE buffer into the gel until the gel is completely covered with buffer. The buffer should just fill the wells. Too much buffer will reduce the current moving through the gel.

PART C: Sample preparation and well loading.

1. Place 15 ul of the cut lambda DNA into a centrifuge test tube.

2. Add 10 ul of loading dye to the DNA in the test tube.

3. Mix by drawing the DNA/dye mixture up and down into the micropipette.

4. Draw 8 ul of the mixture into the pipette. Carefully place the tip of the pipette into the well. (Generally, you should not use the wells at the very edge unless this can't be avoided). Slowly inject the mixture into the cavity. Do not puncture the bottom of the well. Make sure you keep track of which well contains which substance.

5. Repeat step 4 using a different well.

6. Draw 8 ul of the loading dye (not the DNA mixture). Pipette this into another well.

PART D: Electrophoresing

1. Place cover on gel box.

2. Check to insure that the power supply is off.

3. Connect the red cord to the red terminal on the power supply and the red (anode) terminal of the gel box. In the same way connect the black cord to the black terminal on the power supply and the black (cathode) terminal on the gel box.

4. Set the power supply switch to 12 volts.

5. Turn the power supply on.

6. Run the electrophoresis until the bromophenol dye (purple band) of the loading dye mixture is one centimeter from the end of the gel.

7. Turn the power supply off.

8. Disconnect red and black power cords.

9. Remove the chamber cover and remove the gel tray.

PART E: Staining and destaining

1. Carefully slide the gel from the gel tray onto a staining tray. (Styrofoam meat trays work well for this). The gel can be wrapped in Saran and refrigerated overnight if needed.

2. Cover the gel with 0.025% methylene blue stain.

3. Allow to stain for 15 minutes.

4. Pour the stain back into stock solution container.

5. Cover the gel with tap water. Allow to destain for 2 minutes. Pour water off. Add clean tap water. Destain for 2 more minutes. Continue this process until DNA bands become visible. Destaining too long will result in loss of bands.

6. Wrap in Saran and store in the refrigerator overnight. This will further develop the bands.

7. View on white light table, if available.

8. Measure the distance of the DNA bands from the point of origin.

DATA:

Draw a sketch of your gel showing the DNA bands. Be sure to properly label the gel.

QUESTIONS:

1. Explain why some DNA fragments move farther than others.

2. List several factors which affect the distance a molecule will move in gel electrophoresis.

TEACHER'S GUIDE:

MATERIALS:

Lambda DNA - this can be ordered from Sigma Chemical Company or through MSU Biochemistry Stores

Loading Dye - 0.1% bromophenol blue in 50% glycerol

OR

methylene blue stain - 0.025 g diluted in 100 mL of distilled water. A stock solution of 0.1% methylene blue is handy to have in any biology classroom. This is a vital stain and can be used for many purposes. A serial dilution of this will produce the 0.025% stain needed for this experiment.

DISPOSAL:

Gels can be disposed of in a landfill. Buffer can be re-used for future electrophoresis

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