

CH 114
Introduction to Forensic Chemistry
Packet
Spring Term

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Table of Contents

Safety Contract	3
Grade Distribution Form	5
Article Review	7
Evaluating Internet Sources	9
Introduction to the Metric System and Conversions	11
Examination of Glass Fractures	15
Elements of the Periodic Table	17
Atoms of Crime	19
Genotypes and Phenotypes in Blood	21
DNA Replication, Transcription and Translation	25
Amino Acid Codons	27
Measurements and Density Lab	29
Refractive Index of Glass Fragments Lab	33
Determination of Glass Density Lab	35
Observing Chemical Reactions Lab	39
Thin Layer Chromatography Lab	41
Spectroscopy Lab	45
Identification of Drugs and Poisons Lab	49
Restoring Serial Numbers Lab	53
Identification of Unknowns by Infrared Spectroscopy Lab	55
Blood Identification and Typing Lab	59
Visible Spectroscopy Lab	63
Isolation DNA Lab	69
Analysis of DNA: PCR and gel electrophoresis Lab	73
Learning Objectives, Chapter Summaries and Study Guide for Exam #1	79
Learning Objectives, Chapter Summaries and Study Guide for Exam #	81

Please refer to the course calendar for a schedule of labs. The labs will likely be completed in a different order than they appear in this packet.

CH 114 Safety Contract: The chemicals you are using may be hazardous to you and the environment. Dispose of chemicals only as directed by the instructor. If you have questions, please ask your instructor.

- 1) Take small amounts of all chemicals to prevent excess waste. Dispose of all chemicals in the proper waste container. Do not dump chemicals down the drain unless instructed to do so by your instructor.
- 2) Always label the container you use to store chemical reagents. Do not use unlabeled reagents. Adding the wrong reagent could be hazardous and may ruin your experiment so pay attention to your procedure and container labels.
- 3) Never return chemicals to the supply containers, share with a neighbor or dispose of excess chemicals in the waste containers.
- 4) Notify your instructor of chemical spills immediately. Spills need to be cleaned up properly and quickly. Your instructor will tell you the proper method for cleaning up all spills.
- 5) Clean your glassware. Do not put dirty glassware back in your drawer. Do not store chemicals in your drawer without your instructor's permission.
- 6) Please clean up your work area before leaving lab. Return all glassware to its original location.
- 7) If you break a piece of glassware please clean it up using the broom and dust pan in the lab and dispose of broken glass in the white bucket at the front of the lab. Please do not put broken glass in the garbage; this presents a safety hazard to our custodian staff.
- 8) Smoking is not permitted anywhere in the building and horse-play in the lab is dangerous.
- 9) Do not perform any unauthorized experiments. Combining chemicals without your instructor's permission may jeopardize the safety of you and your fellow students.
- 10) Know the location of all safety equipment in the lab, including the safety shower, eye wash, fire extinguisher, first-aid kit and fire blanket. In case of fire or accident, notify your instructor immediately. Evacuate the lab immediately in the case of a fire.
- 11) Do not remove any chemicals from the lab without your instructor's permission.
- 12) Eating and drinking are not permitted in the lab. Please wash your hands before leaving lab to prevent chemical contamination of your next meal or snack.
- 13) Never work alone in the lab, accidents may happen. An instructor or supervisor should be present at all times.
- 14) Dress appropriately for lab. Open-toed shoes, sandals, short skirts or shorts are not permitted in the lab. Long hair should be tied back and contact lenses should not be worn to lab. Safety goggles must be worn in lab at all times.
- 15) Material Safety Data Sheets (MSDS) are available for you to look at for all labs. If you have safety or health concerns please discuss these with your instructor. If you are pregnant, please notify your instructor. Some chemicals used in lab may not be safe for the baby.

I have read these rules and instructions and I shall adhere to them in the lab.

Signed

Date

CH 114 Grade Distribution Form

Name: _____

You have the opportunity to tailor your grade in this class to best suit your learning style. **You can negotiate your grade agreement one time only, at Week 7, but only within the options presented here.**

Labs will be completed weekly, sometimes twice a week. Each lab will be worth 5 to 20 points. This is a lab science course so labs must count between 20 and 30% of your course grade. Late labs will lose 25% per class day late. Students completing labs in groups of two (maximum) may choose to submit one lab report for the group. Each student in the group will receive the same score for the report. Extra credit is possible for labs up to the maximum number of points possible for lab.

In-class exams: CH 114 will have to in-class exams, one during the course near week 6, and the other during finals week. Exams focus on class discussions, online resources posted on Moodle, labs and article discussions. Topics for each exam will be outlined in class. Each exam will count between 20 and 30% of your course grade however each exam does not have to count the same so you may pick which exam you want to count for a greater portion of your grade. Exams may have up to 5 extra points available but no other extra credit for exams is possible.

Small projects, activities, article/reading reviews and writing assignments will be worth between 15 and 25% of your course grade. Many of these assignments will be completed during class time in small groups. No extra credit is possible however in-class activities may be completed independently due to an absence.

Labs will count for _____% of my course grade (20% to 30%).

Exam #1 will count for _____% of my course grade (20% to 30%).

Exam #2 will count for _____% of my course grade (20% to 30%).

Activities will count for _____% of my course grade (15% to 25%).

Total must equal 100%.

CH 114 Article Review Revision (15 points)

- Rough Draft (3 points) is due in class the day the article is discussed and must be initialed by the instructor. Final draft will be due the following class

Introduction (3 points)

- Briefly summarize the article in your own words.
- What topic(s) or questions does the article discuss?
- What background information is provided?
- Briefly summarize one or two of the most interesting points of the article for you.

Forensics and Science (3 points)

- How does the article relate the topic of forensics with science?
- Briefly summarize the supporting data and/or key evidence presented in the article.
- What topics mentioned in the article have been (or will be) discussed in CH 114? Please include the chapter from the text or a related lab or a class date for discussion of the topic.

Evaluating the Source (3 points)

- Briefly evaluate the article using the CRRAPP criteria.
- Is the article popular or scholarly?

Conclusions (3 points)

- What conclusions or points do the article authors make?
- What questions did the article raise but not answer?
- How might you be able to answer these questions?

CH 114 Evaluating Internet Sources

Watch the video at: https://library.lanecc.edu/howtos/video/evaluating_internet_sources

Are the following statements True or False?

- 1) Educational and governmental websites are more likely to be biased sources of information.
- 2) Shortening the website's url can help provide more information about sponsors of the site or organizations aligned with the site.
- 3) Different search engines will give different results.
- 4) Only the first few results should be reviewed to get a thorough understanding of the topic.

Short Answer:

- 5) What questions should be asked when evaluating a website?

- 6) What does CRRAPP stand for?

CH 114 Introduction to the Metric System and Unit Conversions

As mentioned in class, the metric system is the unit of choice in science labs. The metric system uses prefixes combined with a base unit. The base unit for length is the meter. The base unit for mass is the gram and the base unit for volume is the liter. The metric system uses other base units as well but the gram, liter and meter are the three main units we will be working with this term. A derived unit forms when a prefix combines with a base unit. A derived unit is chosen based on the size of the measurement. For example, the milli prefix represents 1/1000 or 0.001 base units. One meter contains 1000 millimeters or 1 millimeter equals 0.001 meters. If you were measuring the distance between your house and the college, millimeters would not be an appropriate unit to use because they are so small. Instead, using meters or more likely kilometers would be a more appropriate choice. Some of the more common prefixes (i.e. the ones we will use in class) are listed below:

<u>Prefix</u>	<u>Symbol</u>	<u>Numerical Value</u>
kilo-	k	1000 base units = 1 kilo-
centi-	c	100 centi- = 1 base unit
milli-	m	1000 milli- = 1 base unit
nano	n	1*10 ⁹ nano- = 1 base unit

Units of measurement are abbreviated using symbols for both the prefix and the fundamental unit. A meter is represented by the symbol m, a liter is represented by L and a gram is represented by g.

Often the unit of a measurement will need to be converted into another unit (either a metric unit or an English unit used in the United States). Cookbooks represent an excellent source of unit conversions. Listed below are some common conversions. You will not need to memorize any conversions in this course. If you can't find a conversion you need, please ask.

Helpful Unit Conversions for CH 114

1 inch = 2.54 centimeters	1 meter = 39.37 inches
1 foot = 30.5 cm	1 liter = 1.06 quarts
1 pound = 453.6 grams	1 kilogram = 2.2 lbs
1 ounce = 28.35 g	1 qt = 0.94 L
1 fluid ounce = 29.6 mL	

Units can be converted using a process called the factor labeled method. This problem solving skill is helpful for familiar unit conversions like these but can also be used for conversions between units we aren't as familiar with. In order to convert the given units to the desired units we need to multiply the given units by a conversion factor:

$$\text{given units} * \text{conversion factor} = \text{desired units}$$

Notice that all of the unit conversions listed above include two different units and therefore we can treat each as a fraction where we decide which unit goes in the numerator and which unit goes in the denominator. We multiply numbers and units in the numerator and divide by numbers and units in the denominator.

$$\text{given units} * \frac{\text{desired units}}{\text{given units}} = \text{desired units}$$

The given units in the above generic example cancel out (because we are dividing by the given units in the denominator of the unit conversion) leaving the desired units. Let's look at a more specific example.

Example: One of the footprints from the unknown animal or bird was measured to be 0.50 cm, how long was the print in millimeters?

This question is asking us to convert centimeters to millimeters so we need to look up (or know) that conversion. From the chart above, we know there are 100 mm in 1 cm and 1000 mm in 1 m so that means there are 10 mm in 1 cm (we could also count the small gradations on the ruler too). Our given unit is cm and our desired unit is mm.

$$0.50 \text{ cm} * \frac{10 \text{ mm}}{1 \text{ cm}} = 5.0 \text{ mm}$$

The cm units cancel out top and bottom and we are left with only mm. We can check our work by inverting the unit conversion:

$$0.50 \text{ cm} * \frac{1 \text{ cm}}{10 \text{ mm}} = 0.05 \text{ cm}^2/\text{mm}$$

Notice the mess of units we got from this set up. Instead of the cm canceling out, they are multiplied together. Doing the math with your units, as well as the numbers is an excellent way to check your work and be confident you solved the question correctly.

What happens when a single unit conversion isn't available? Now we need to string together two or more unit conversion as shown below.

Example: A bullet slug has a mass of 0.75 ounces. What is the mass of the slug in kilograms?

From the charts above we know that 1 ounce = 28.35 g and 1000 g = 1kg. Our given unit is ounces and our desired unit is mg.

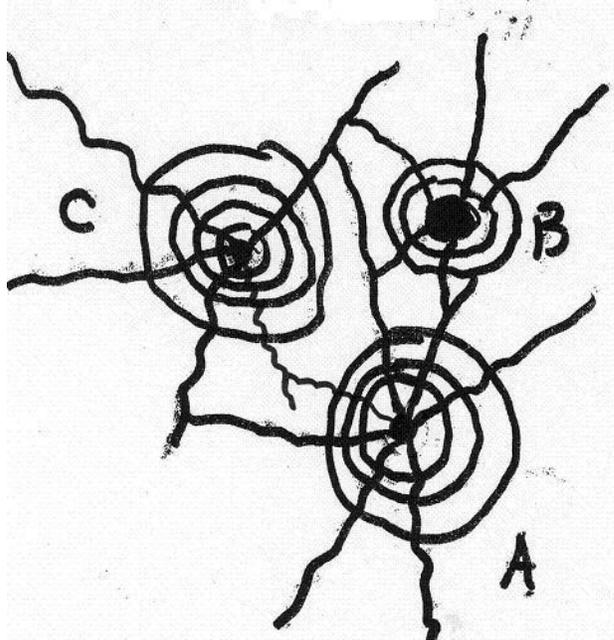
$$0.75 \text{ oz} * \frac{28.35 \text{ g}}{1 \text{ oz}} * \frac{1 \text{ kg}}{1000 \text{ g}} = 0.021 \text{ kg}$$

CH 114 Examination of Glass Fractures

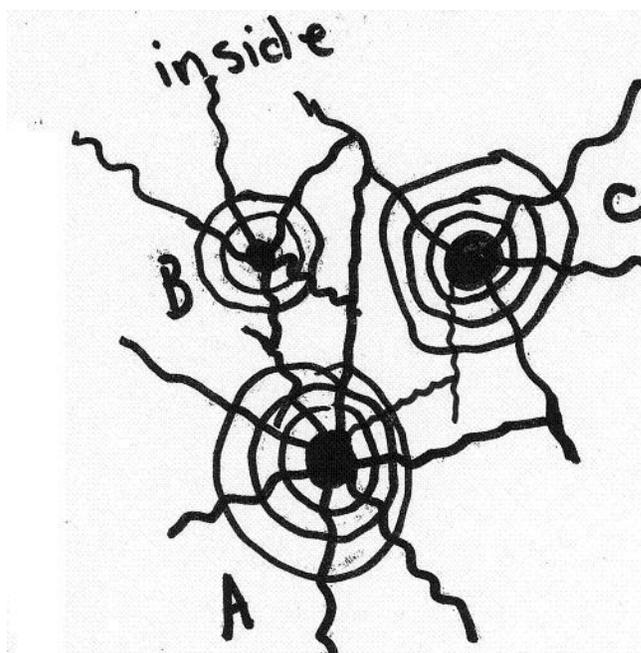
The goal of this activity is to examine representations of fractured glass to determine the direction of impact and the sequence of bullets.

Late in the evening, Mr. and Mrs. Lane are in the kitchen getting a bedtime snack and discussing the rise of violence in the neighborhood. The number of burglaries has been steadily increasing since the New Year began. Mr. Lane tries to reassure Mrs. Lane, reminding his wife that he has just put new locks on all the doors and bought a gun for each room in the house, including the kitchen. Mrs. Lane would prefer a watch dog instead of having so many guns in the house and she isn't shy about telling Mr. Lane. With bed time snack in hand, Mrs. Lane heads upstairs to read before bed. Just as she reaches the top of the stairs she stops at the sound of three quick gunshots. She races downstairs to find Mr. Lane standing in the kitchen with a gun in his hand and three bullet holes in the newly installed kitchen window.

Examine the representations from the inside and outside of the Lane's window on the back of this page and determine from which direction the bullets were fired and in what sequence. Briefly explain your reasoning for each in the space below.



Outside



- 4) The criminal act of deliberately setting fire to property is a five-letter word and can be spelled by the combined symbols of four elements forward or three elements spelling it backwards. The forward spelling includes a noble gas and three nonmetals, while the backwards spelling includes a lab-generated actinide series element, a nonmetal and a radioactive alkaline earth metal.
- 5) The combined symbols of a nonmetal, a radioactive halogen and a lab made lanthanide series element give the ___-logy of forensics involving the investigation of sudden, unnatural, unexplained or violent death.

CH 114 Atoms of Crime

- 1) In the Elements of the Periodic Table activity, question 2 asked you to combine one transition metal and one nonmetal to form the acronym for the analytical technique we used to analyze the ink samples in lab.
 - a) What was the acronym?

 - b) Name the elements used to spell the acronym.

 - c) What is the sum of the protons in the two elements?

- 2) Napoleon was thought to have been poisoned. See the following website for details: <http://news.nationalgeographic.com/news/2007/01/070117-napoleon.html>
 - a) What is the name and symbol of the element thought to have poisoned Napoleon?

 - b) If an atom of that element contained 41 neutrons, what is the atomic mass of the element?

- 3) The bullets used in the Kennedy assassination have been reexamined in 2007. The results are published in The Annals of Applied Statistics, 2007, Vol 1, No. 2, 287-301. <https://arxiv.org/pdf/0712.2150.pdf>
 - a) What are the names and symbols of the two elements whose concentration were analyzed in the bullets according to Table 1 of the article?

 - b) How many protons, neutrons and electrons does each element contain? Use the mass of the most common isotope.

- 4) In 2006, ex KGB office Alexander Litvinenko was deliberately poisoned with polonium-210 (someone spiked his tea). Read more about at <http://www.bbc.com/news/uk-19647226>. Polonium is a radioactive element. It releases alpha particles until it becomes lead. The release of alpha particles inside living cells disrupts the cells leading to death.
- a) How many protons, neutrons and electrons are in polonium-210?

 - b) Alpha particles are the same as a helium nucleus (mass = 4). How many protons and neutrons are in an alpha particle?

 - c) How many protons, neutrons and electrons are in an atom of lead-208?
- 5) Most of the naturally occurring carbon is carbon-12. Two other isotopes of carbon exist, carbon-13 and carbon-14. Carbon-14 is used in radioactive carbon dating to estimate the age of artifacts made from wood or cloth.
- a) How do the isotopes of carbon differ from each other?

 - b) How are the isotopes of carbon alike

Genotypes and Phenotypes in Blood

- 1) The following table shows the blood type of mothers and children (lots of different families are shown). Please complete at a minimum **half** of the following table being sure to list all possibilities in each box. Once you have listed **possible** genotypes for the mother and child, list possible blood types and genotypes for the father, then **impossible** genotypes for the father (genotypes that can be eliminated as possibilities). As extra practice you may also wish to list the impossible father's blood type.

Mother Blood Type	Mother Possible Genotypes	Child Blood Type	Child Possible Genotypes	Father Possible Blood Type and Genotypes	Father Impossible Genotypes	Father Impossible Blood Type
A	AA or AO	O	OO only	A or B or O (AO or BO or OO)	AB, AA, BB	AB
B		AB				
O		A				
AB		A				
O		O				
B		B				

Mother Blood Type	Mother Possible Genotypes	Child Blood Type	Child Possible Genotypes	Father Possible Blood Type and Genotypes	Father Impossible Genotypes	Father Impossible Blood Type
AB		AB				
A		AB				
B		A				
AB		B				

Use the space below to show a minimum of three Punnett squares for your responses listed in the table above.

- 2) People's Court: A Paternity Test. Mr and Mrs Jones had a baby boy named Skippy. The Jones' neighbor, Mr. King claimed he was the father of Skippy. Blood tests were conducted with the following results:

Person	Blood Type	Possible Genotypes
Skippy	O	
Mrs. Jones	B	
Mr. Jones	AB	
Mr. King	A	

- a) You are the judge (wow, law school went quick!). How would you rule? Please explain your answer clearly.

- b) How would your ruling change if Skippy's blood type was AB, instead of O? Please explain your answer clearly.

- 3) If you can't make a determination in either case, what do you suggest happen next? Be sure your answer relates to forensic chemistry class. In other words, what other tests might you recommend?

- 2) Gln-His-Pro-Gly is a sequence known as progenitor thyrotropin-releasing hormone (pro-TRH). If the pro-TRH gene was being used to type DNA, its base sequence would be needed.

Gln-----His-----Pro-----Gly

- a) Given the sequence of amino acids above, complete the grid below with the **mRNA** sequence for the pro-TRH gene:

5'													3'
----	--	--	--	--	--	--	--	--	--	--	--	--	----

- b) Using your answer to part a above, complete the grid below with the **DNA template strand**. Remember this strand goes in the opposite direction (from 3' to 5' instead of 5' to 3')

3'													5'
----	--	--	--	--	--	--	--	--	--	--	--	--	----

- c) Lastly, using your answers to previous questions, complete the grid below with the DNA informational strand.

5'													3'
----	--	--	--	--	--	--	--	--	--	--	--	--	----

- d) Which strand above represents the sequence of bases in the original strand of DNA?

- e) Would the pro-TRH gene ever be used in a forensic context to type DNA? Briefly explain your answer.

Amino Acid Codons

UUU	phenylalanine	UCU	serine	UAU	tyrosine	UGU	cysteine
UUC	phenylalanine	UCC	serine	UAC	tyrosine	UGC	cysteine
UUA	leucine	UCA	serine	UAA	terminator	UGA	terminator
UUG	leucine	UCG	serine	UAG	terminator	UGG	tryptophan
CUU	leucine	CCU	proline	CAU	histidine	CGU	arginine
CUC	leucine	CCC	proline	CAC	histidine	CGC	arginine
CUA	leucine	CCA	proline	CAA	glutamine (Gln)	CGA	arginine
CUG	leucine	CCG	proline	CAG	glutamine	CGG	arginine
AUU	isoleucine (Ile)	ACU	threonine	AAU	asparagine (Asn)	AGU	serine
AUC	isoleucine	ACC	threonine	AAC	asparagine	AGC	serine
AUA	isoleucine	ACA	threonine	AAA	lysine	AGA	arginine
AUG	start (methionine)	ACG	threonine	AAG	lysine	AGG	arginine
GUU	valine	GCU	alanine	GAU	aspartic acid	GGU	glycine
GUC	valine	GCC	alanine	GAC	aspartic acid	GGC	glycine
GUA	valine	GCA	alanine	GAA	glutamic acid	GGA	glycine
GUG	valine	GCG	alanine	GAG	glutamic acid	GGG	glycine

- each amino acid is abbreviated with the first three letters of the amino acid name
 - for example, phenylalanine is abbreviated Phe
- the first letter is capitalized
- some amino acids have a different abbreviation, those are noted in () following the amino acid name
 - isoleucine is abbreviated Ile

Measurements and Density Lab

The purpose of this lab is to learn how scientific measurements are carried out properly using common measuring instruments. In this experiment the density of solid and a liquid will be determined. This lab will also serve to demonstrate the use of significant figures in calculations and allow both the accuracy and precision of the measurements to be evaluated.

Procedure

A. Density of ethanol

- 1) Determine the mass of a clean, dry 50-mL Erlenmeyer flask fitted with a rubber stopper. Record the mass of the flask on the data sheet to the proper number of significant figures (this means you should record all the numbers from the balance).
- 2) Pipet 25.00 mL of ethanol into the weighed flask and replace the stopper to seal the flask.
- 3) Determine the mass of the flask and the ethanol on the same balance used in step 1. Record the mass to the proper number of significant figures on the data sheet.
- 4) Calculate the mass of the ethanol by subtracting the mass of the empty flask from the mass of the ethanol and the flask.
- 5) Use the mass of ethanol and the volume of ethanol to calculate the experimental density of ethanol to the correct number of significant figures.
- 6) Using a thermometer, determine the temperature of the ethanol in the flask. Record the temperature on the data sheet.
- 7) Record the accepted density of ethanol.
- 8) Use the accepted density of ethanol and the experimental density of ethanol to calculate the absolute error, relative error and percent error. See the data sheet for the required equations.

C. Density of a metal slug

- 1) Obtain a metal slug and record its identity (color description or number) on the data sheet.
- 2) Determine the mass of the slug. Record the mass on the data sheet to the correct number of significant figures.
- 3) Determine the volume of the metal slug using a ruler to measure the diameter and height of the slug. Record your measurements. Using the mass and the volume of the metal slug, calculate the density of the slug and report your answer to the correct number of significant figures.

D. Density of a metal slug using water displacement

- 1) Partially fill a graduated cylinder with water. Record the initial volume of water in the cylinder.
- 2) Gently slide the metal slug into the cylinder being careful not to break the bottom. Be sure the slug is completely submerged. Record the final volume of the water.

Data Sheet

A. Density of ethanol

Volume of ethanol: _____

Gross weight (sample + container): _____

Tare weight (container): _____

Net weight (sample): _____

Calculate the experimental density of ethanol in the space below. Show all your work. Report the density with four digits.

Experimental density of ethanol = _____

B. Error Calculations

The density of many substances varies with temperature. Use a handbook to determine the accepted density of ethanol at the temperature measured in the lab.

Temperature of ethanol: _____

Accepted density of ethanol: _____

Experimental density of ethanol: _____

Calculate the percent error your experimental measurement by subtracting the accepted value from the experimental value and dividing by the accepted value, then multiply by 100. Ignore the negative sign, it just means your experimental value was less than the accepted value.

C. Density of a metal slug

Identity of the metal: _____

Mass of slug: _____

Diameter of slug: _____

Height of slug: _____

In the space below, calculate the volume (V) of the slug. Show all your work. $V = (\pi * \text{diameter}^2 * \text{height}) / 4$

In the space below, calculate the density of the slug. Show all your work and include units. Report the density with three digits.

D. Density of the slug using water displacement

Mass of slug: _____

Final Volume of water: _____

Initial Volume of water: _____

Volume of slug: _____

The volume of the slug is equal to the final water volume-initial water volume.

Refractive Index of Glass Fragments Lab

Refractive index is an intensive property meaning it can be used to characterize an unknown because all materials have their own characteristic index of refraction. Refractive index is discussed in detail in chapter 4 of the text (1). Determination of the refractive index of a glass fragment may provide additional information to the forensic scientist. The density of a glass fragment is usually determined first and the index of refraction used as a confirmatory test. By comparing the index of refraction of a glass fragment collected at a crime scene with another sample perhaps collected from a suspect, the forensic scientist may be able to determine whether or not the two fragments have a common source. Just as important, by comparing the fragments the forensic scientist may be able to disprove the possibility of the fragments having a common source. If density determinations and index of refraction comparisons indicate a common source, the frequency of occurrence of refractive index values may be used to assess the evidential value of the glass.

The refractive index of glass can be experimentally determined in a lab using a refractometer or the immersion method. In this lab we will use a simplified version of the immersion method. When a sample of glass is placed in a liquid with the same index of refraction, the piece of glass disappears because light travels through both the glass and the liquid at the same speed. Light travels in air at the speed of approximately 3.0×10^8 m/s but when it penetrates an object such as glass it slows down. This change in velocity of the light causes the light to bend or refract. A Becke line is observed near the border of a particle immersed in a liquid with a different index of refraction. If the particle and the liquid have the same index of refraction no Becke line is observed.

In this experiment we will immerse several known and unknown samples of glass in liquids with different indices of refraction for comparison. The table below lists the index of refraction for some common types of glass (2).

<u>Glass</u>	<u>Index of Refraction</u>
<u>Headlight</u>	<u>1.47-1.49</u>
<u>Television</u>	<u>1.49-1.51</u>
<u>Window</u>	<u>1.51-1.52</u>
<u>Bottle</u>	<u>1.51-1.52</u>
<u>Ophthalmic lenses</u>	<u>1.52-1.53</u>

Procedure (2)

Carefully (so as to not cut yourself) place each known and unknown sample in the liquid assigned to your lab group. Label each beaker with the glass identity. Use the 50-mL beaker and only add enough of the liquid to cover the piece of glass. The liquids are Wesson oil (index of refraction of 1.47), clove oil (index of refraction of 1.5430) and a 3:1 mixture of clove oil and Wesson oil (index of refraction of 1.52). The index of refraction for olive oil is 1.467, which is very similar to Wesson oil so we will only test the Wesson oil.

On a separate sheet, record your observations of the physical properties of each type of glass and for each sample in the oils. Use a \checkmark if you can see the glass fragment and an X if you cannot see the glass fragment. A sample table is shown below.

Glass Fragment	Physical properties	Wesson Oil	Clove Oil	Wesson/Clove
Window				
Unknown #				

Please answer the following questions on the bottom of this page or a separate sheet.

- 1) What conclusions can you draw from your results? Did your unknown glass sample have the same index of refraction as any of the liquids? Briefly explain.
- 2) Does your unknown have a common source with any of the known samples? How certain are you of your conclusions? Please explain.
- 3) What other information would be helpful for you to determine whether two fragments of glass have a common source?
- 4) The index of refraction of pyrex is not listed in this lab. Based on your experimental results, what is the approximate index of refraction of pyrex. Briefly explain.
- 5) Which of the following liquids used in this lab would television glass not be visible in? Briefly explain.

Literature Cited

- 1) Saferstein, Richard; *Criminalistics*, 9th ed.; Pearson Prentice Hall: New Jersey, 2007.
- 2) Meloan, Clifton E.; James, Richard E.; Saferstein, Richard; *Criminalistics Lab Manual*, 9th ed.; Pearson Prentice Hall: New Jersey, 2007.

Determination of Glass Density Lab

Glass is commonly found at crime scenes. Density, like refractive index is an intensive physical property. The characterization of glass using density is therefore a key aspect of forensic science. According to our text “at this time, the physical properties of density and refractive index are used most successfully for characterizing glass particles” (1). By determining and comparing the density of two different samples of glass (one from the crime scene and the other from a suspect for example), the forensic scientist may be able to determine if the samples share a common source. The greatest evidential value comes from assembling and physically piecing together glass fragments collected at the crime scene and from the suspect (making a physical match). In the absence of a physical match, the determination of density begins the process of establishing the possibility or impossibility of the glass fragments having a common origin.

Forensic labs will compare glass samples using a density gradient column or a comparative technique called flotation. We will use a density gradient to analyze soil samples later this term and will save our discussion of this technique until that time. In the flotation technique a fragment of glass is placed in a sample of liquid with a known density. If the glass fragment sinks in the liquid, then the density of the fragment is greater than the density of the liquid. If the glass fragment floats on the liquid then the density of the liquid is greater than the density of the glass. If the glass fragment is suspended in the liquid then the densities of the glass and the liquid are equal (just like in a Galileo thermometer). The flotation technique begins with a sample of unknown glass being added to a dense liquid such as bromoform with a density of 2.89 g/mL. If the glass fragment floats then a less dense liquid such as bromobenzene ($d = 1.52$ g/mL) is added until the glass fragment is suspended in the liquid. A similarly sized fragment of known glass is then added to the same liquid for comparison. If the known and unknown samples have similar densities they will both be suspended in the liquid. If the samples have different densities, one will be suspended in the liquid and the other will sink or float.

The flotation technique is more commonly used for comparing two glass samples (one from the scene, the other from the suspect) however the chemicals required are toxic to both people and our environment and therefore will not be used for our class. Instead we will experimentally determine the density of known and unknown samples of glass using Archimedes's principle (it's the same principle governing flotation). This technique would be useful if only one glass fragment is available (in other words suspect and scene samples do not exist for comparison) but can also be used for making comparisons.

We have already determined the density of various objects in a previous lab. In this lab, we will determine the mass of a fragment of glass using an electronic balance. The determination of volume for an oddly shaped piece of glass is more complicated. Water displacement may work if the fragment will fit into a graduated cylinder. What happens if the fragment won't fit? We could break it but that seems dangerous while also altering potential evidence. Instead we will use a physics relationship called Archimedes's principle. Archimedes's principle states that an object immersed in a fluid displaces a volume of fluid equal to the object's volume (2). Stated another way, an object immersed in a fluid is buoyed up by a force equal to the weight of the displaced

fluid (2). The volume of an object suspended in water can be determined by weighing the object in water. The object will displace a volume of water equal to the object's volume. Because water has an approximate density of 1 g/mL, the mass of the object in water equals its volume. Let's look at an example: the mass of a fragment of glass suspended on a string over a balance has a mass of 6.57 g in air and a mass of 2.88 g in water. The volume of the object is equal to $2.88 \text{ g} \cdot 1\text{mL}/1\text{g} = 2.88\text{mL}$. The calculated density of the glass is $6.57 \text{ g} / 2.88 \text{ mL} = 2.28 \text{ g/mL}$, fairly close to the literature density of window glass of 2.47 to 2.56 g/mL (we'll have to think of some sources of experimental error to explain our low density!). This procedure can be adapted for any irregularly shaped solid when the water displacement method will not work. Please note: because our glass fragment will be suspended over the balance, the fragment's weight in water equals its volume.

Procedure:

- 1) Obtain a piece of string, 400-mL beaker and a known glass fragment. Be sure to keep the glass fragments separated and/or clearly labeled, we don't want to switch the samples.
- 2) Carefully tie the string around the glass fragment. Trim off any excess string near the knot.
- 3) Suspend the glass fragment on the string over a triple beam balance. Use a clamp for the support.
- 4) Measure and record the mass of the glass fragment and string to two decimal places
- 5) Partially fill a 400-mL beaker with water.
- 6) Place the beaker of water on the same triple beam balance and record its mass to two decimal places.
- 7) Suspend the glass fragment on the string in the water. Be sure the glass is completely submerged but is not touching the sides of the beaker. Measure and record the mass to the correct number of significant figures.
- 8) Repeat with an unknown sample. The mass of the beaker and water also needs to be remeasured because the string from previous trials will absorb water.
- 9) Calculate the density of each sample and record your results on the board to share with the rest of the class.
- 10) Look up the acceptable density range for each type of glass. If the experimental density is not within 1 g/mL of the accepted value, repeat the measurements.
- 11) Return all materials, clean and dry your equipment.

Optional Procedure: If your sample of glass will fit in a graduated cylinder you may use water displacement to determine the volume of the sample. Please follow the procedure used during lab 1.

Data and Calculations: Record on a separate sheet. A sample table is shown below. The mass of glass in water and the volume of glass are equal so the far right two columns have the same values.

Glass Fragment	Mass with string in air (g)	Mass of beaker and water (g)	Mass of beaker, water and glass (g)	Mass of glass in water(g)	Volume of glass (mL)
Window					
Unknown #1					

Calculate the density of each of your glass fragments. Please show your work on the following page and report your results to the instructor or on the board.

Conclusions: please answer the following questions on the back of this page or a separate page.

- 1) What conclusions, if any, can you make from the experimental results? Briefly explain. Can you determine if your unknown shared a common origin with any of the known samples?
- 2) Which method did you use to determine the density of glass, Archimedes's principle or volume displacement? Which method was the most accurate and/or precise? If you used only one method, please briefly explain why.
- 3) Window glass has a density of 2.47 to 2.56 g/mL. How do the experimentally determined densities for window glass compare?
- 4) Briefly explain two sources of experimental error for this lab. Your lab partner and errors in calculations are not options here!
- 5) Briefly explain how the flotation technique works, why this technique is useful and why it's not being used in CH 114.

Literature Cited

- 1) Saferstein, Richard; *Criminalistics*, 9th ed.; Pearson Prentice Hall: New Jersey, 2007.
- 2) Meloan, Clifton E.; James, Richard E.; Saferstein, Richard; *Criminalistics Lab Manual*, 9th ed.; Pearson Prentice Hall: New Jersey, 2007.

Use this page to prepare a data table, record your data, and show your work for density calculations.

Observing Chemical Reactions Lab

Much of the chemical work in a forensic crime lab involves the analysis of drugs. A suspected drug sample will undergo a presumptive screening test first. Screening tests are nonspecific and preliminary in nature and often include subjecting the unknown material to a series of color tests. Color tests produce a characteristic color for commonly encountered illicit drugs. Even negative results of color tests may eliminate certain drugs from consideration (1). Once the potential class of drug has been determined, confirmatory tests are completed. A confirmatory test is a single test that specifically identifies a drug. In a future lab we will use presumptive color tests to determine the class of drug and then use infrared spectroscopy as a confirmatory test.

Color tests produced a characteristic color when a drug is brought into contact with a specific chemical reagent. Color tests qualitatively demonstrate a drug's chemical properties. Drugs like other common household products such salt, shampoo, water, etc. are chemicals that react or combine with chemical reagents. During a chemical reaction a color change, a temperature change, the formation of bubbles, and/or the appearance of cloudiness may be observed. The formation of bubbles indicates the presence of a gaseous product and the appearance of cloudiness indicates the formation of an insoluble precipitate. Precipitates do not dissolve in water. In this lab, each type of observation of a chemical reaction will be demonstrated.

Safety: Use small quantities of all materials and dispose of all waste in waste containers. Wear safety goggles at all times. Gloves are recommended. Do not taste any reactants.

Procedure

Please note: concentrations are given for many solutions in this lab. The concentration is measured in molarity (M). Please read the label carefully for each step to ensure you are adding the correct reagent at the necessary concentration. Some reactions use the same reagent at a different concentration.

Neutralization: acid + base:

- 1) Place 2 mL of 0.1 M sodium hydroxide, NaOH in a test tube.
- 2) Add 2 to 3 drops of universal indicator.
- 3) Place 2 mL of 0.1 M hydrochloric acid, HCl, in a separate test tube.
- 4) Add 2 to 3 drops of universal indicator.
- 5) Mix the two solutions by pouring one into the other test tube.
- 6) Record your observations. Can you feel a change in temperature?
- 7) Dispose of waste in waste container.

Aluminum in acid:

- 1) Place a few small pieces of aluminum in a test tube and add 2 mL of 2M hydrochloric acid (HCl).
- 2) Record your observations.
- 3) Dispose of waste in waste container.

Base + Copper (II) sulfate:

- 1) Place 2 mL of copper (II) sulfate, CuSO_4 in a test tube.
- 2) Add 0.1 M sodium hydroxide, NaOH , drop wise until you see a noticeable change that does not disappear with stirring.
- 3) Record your observations.
- 4) Dispose of waste in waste container.

Aluminum in copper (II) sulfate:

- 1) Clean a few small pieces of aluminum with steel wool and place them in a test tube. You may also, briefly dip the aluminum in acid.
- 2) Add 2 mL of copper (II) sulfate, CuSO_4 .
- 3) Record your observations.
- 4) Dispose of waste in waste container.

Observations: record your observations in the table below for each reaction and indicate which of the four observations typical of a chemical reaction (color change, temperature change, bubbles or cloudiness) you saw in the Example of: column.

Reaction	Observations	Example of:
Aluminum in acid		
Aluminum in copper (II) sulfate		
Neutralization		
Base + Copper (II) sulfate		

Literature Cited

- 1) Saferstein, Richard; *Criminalistics*, 9th ed.; Pearson Prentice Hall: New Jersey, 2007.

Thin Layer Chromatography Lab

Chromatography is a technique used to separate and identify components of a mixture. Chromatography has been used to identify components of dyes, inks, paints, and chemical compounds in industry, as well as for many other practical uses. All forms of chromatography involve distributing the substance to be identified between two phases, one moving and one stationary. Different components of a mixture move at different rates along the stationary phase because of differing attractions of the components to the stationary phase.

In thin layer chromatography, the stationary phase is a porous medium with a very large surface area. The porous medium may be cellulose, filter paper or a thin coating of silica or alumina on a plastic or glass sheet. The TLC plate is placed in an appropriate liquid solvent. The solvent is drawn up the sheet by capillary action, acting as the moving phase. As the solvent moves up the TLC plate, components in the mixture are also drawn up the plate.

As the TLC plate is developed, certain components of the mixture will stop moving along the plate. The components stop moving because of similar attractions that exist between the stationary and moving phases. If the stationary phase is very polar and the moving phase non polar, then polar components are attracted to the stationary phase and don't move very far up the plate. Non polar components of the mixture are not attracted to the plate, but are attracted to the moving phase, and therefore are carried further up the plate. The different attractions of the components of the mixture to each phase cause the components to separate.

The distance a component of a mixture travels compared to the developing solvent is called the retention factor (R_f). The retention factor is calculated by dividing the distance the component traveled by the distance traveled by the solvent, called the solvent front.

In some cases, components of a mixture are not visible by the naked eye. One method used to expose the developed plate is to examine the plate under ultraviolet light. The UV light will cause some spots on the developed plate to fluoresce. Another method for observing a developed plate is to coat the plate with a compound that will react with the spots to make them visible. For this lab experiment, many of the components of a sample of ink are visible with the naked eye.

In order to identify the unknown ink, a sample of known ink must be separated on the same TLC as the unknown. This procedure has been used in the past to identify a pen used to make notes in a lab book. A scientist was suspected of altering her data to gain financial support. The investigating authorities suspected the scientist had gone back and altered her results. To help in the investigation, the authorities took a sample of writing from the notebook from several different dates. Analysis of the writing samples by TLC could indicate if a different pen was used. Although the results of the TLC experiment would not provide enough data to support or refute the claim, the results were entered into evidence against the scientist.

General Procedure for Thin Layer Chromatography

A. Preparation of the Developing Chamber

- 1) Obtain one jar with lid or one large beaker (400-600 mL) and a piece of foil to cover.
- 2) Add enough solvent to give a thin layer of solvent on the bottom of the beaker. The solvent used to separate the components of ink is 75% ethanol, 25% water.

B. Preparation of Ink Samples

- 1) Obtain a small, but dense ink sample from each pen, including the unknown. Cut the sample into small pieces and transfer to well plate. Clearly label each well with the pen's brand name.
- 2) Add drops of 75% ethanol to each ink sample. Add enough liquid to just cover the paper. Do not add too much liquid otherwise the ink sample will be too dilute. Use the glass capillary to push the paper to the bottom of the test tube. Allow 10-15 minutes for ink extraction. The ink samples should be dark in color. If not, add more ink sample.

C. Preparation of TLC Sheets:

- 1) Prepare the necessary number of TLC plates. The unknown must be run on each plate. Handle the plates only by the edges to prevent contamination by oils on your fingers.
- 2) Lightly draw a line using a pencil 1-cm from the bottom of each plate. On the line make enough small marks for each sample to be spotted on the plate plus the unknown. Space each mark about 1 cm apart so the samples will not run into each other as the plate develops. Label each spot with pencil.
- 3) Spot a sample of each known and unknown on the appropriate mark. Use a different capillary tube for each application. Practice your technique first. For the best results, the applied spots should remain as small as possible. Gently blow on the applied samples to dry each spot. You must be able to see each spot before developing the plate. Several applications will likely be needed. Keep the spots as small and dark as possible.
- 4) Once a sample of each compound has been applied to the TLC sheet, gently place the sheet, origin side down in the appropriate developing chamber. Be sure the solvent level is not higher than the applied spots.
- 5) Develop the sheet. Remove the TLC sheet from the chamber when the solvent is approximately 2 cm from the top of the plate or the spots have separated from each other.
- 6) Immediately mark the solvent front with a pencil. Allow the sheet to dry.
- 7) Observe the plates under an UV lamp and gently circle the location of each spot.

D. Application of Ink directly on the plate

Prepare one additional plate and spot each ink sample directly on the plate. Develop as directed above. Record your observations.

Data and Observations

On a separate sheet of paper record your data in a table or neat list.

Attach your TLC plates, make a drawing of each plate or include a photograph of the plates. Be sure to clearly identify each ink sample and the color of each spot on your drawings.

For each plate, measure and record the distance the solvent moved, called the solvent front. Measure from the origin (where the spots were applied) to the marked solvent front.

For each colored spot for each ink sample, measure and record the distance traveled by each spot.

Calculate the R_f for each spot for each ink sample.

Based on your developed TLC plate, identify the unknown ink? Support your answer with experimental evidence.

Questions: please answer these questions at the bottom of this page or a separate sheet of paper.

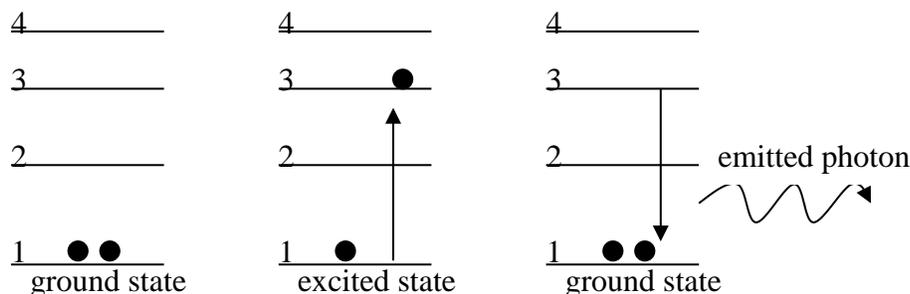
- 1) If two components have an identical R_f value, does this mean they necessarily have the same structure? Explain why or why not.
- 2) Why must an unknown sample be spotted on the same TLC plate as the known sample?
- 3) Would TLC work for separating waterproof inks? Briefly explain.
- 4) Why must the spot applied to the plate be small?
- 5) Could the procedure of spotting the ink directly on the plate be used to identify if an unknown sample shares a common source with a known sample? Briefly explain.

Spectroscopy Lab

When white light from an incandescent lamp is passed through a prism, it produces a continuous spectrum of colors. We see this often in Oregon in the form of the rainbow that is formed when sunlight passes through a matrix of raindrops. The different colors of light represent different wavelengths. Blue light has a shorter wavelength than red light. Pure white light is a combination of all the various colors.

If the light from a gas discharge tube that contains a particular element is passed through a prism, only narrow colored lines are observed as opposed to the continuous spectrum we see in a rainbow. Each line corresponds to light of a particular wavelength. The pattern of lines emitted by an element is called its line spectrum. Each element has its own characteristic spectrum. Emission spectroscopy is therefore an important analytical technique useful for the forensic chemist trying to identify the elements present in an unknown material.

An electron can be bumped up to a higher orbital by absorbing a quantum of energy—one way to do this is to heat the atom. After a while of “orbiting” at a higher level, the electron will spontaneously fall back to a lower energy level. In doing this, the electron is going to have to give up a unit of energy in the form of a photon.



Every atom of a specific element always has its electron orbitals in the same place. Hence, in the modeled atom above, every time an electron falls from the 2nd excited state to the ground state, we are going to get a particular frequency of light out.

As every element has a different electron orbital structure, we should be able to identify a substance by the characteristic spectral lines that are emitted when we expose it to high heat or electrical current.

In a flame test, electrons of a metal ion are excited using heat, just as electrons are excited in a gas discharge lamp. Flame tests are often used to identify unknown metals. When certain metals burn in a flame, they produce a colored flame characteristic of the metal. Certain colors of fireworks are produced using specific metals. In this portion of the experiment a spectroscope will not be used, as a result a line spectra will not be observed.

Procedure:

1. Spectra of Gases:

A tube of hydrogen gas will be excited via an alternating high-voltage electrical current. Using a spectroscope, you will examine the characteristic wavelengths of light emitted. For hydrogen, you may see four distinct lines. The violet line may not be visible to all people.

Record in the attached table the color and relative intensity of each visible line by drawing what you see. Repeat the process for the other 5 elements.

2. Flame Tests of Known Compounds:

- a. Mount a Bunsen burner on a ring stand so that the burner is held at about 45° angle from perpendicular. We do this so that material won't drop into the Bunsen burner. Place a hot pad under the burner to prevent drops of molten salts burning into the tops of the laboratory counters.
- b. Observe flame test colors produced by each of the known compounds in the flame test kit. To prevent contamination, keep flame test wires and sample together—
DO NOT SWITCH FLAME TEST WIRES FROM ONE SAMPLE TO ANOTHER!
- c. Record the color of each flame test on the attached data table.

3. Flame Tests of Unknown Compounds:

Choose two unknowns (only one unknown may be blue in color) and determine what metal each unknown contains. Flame tests can only be used to identify possible metals. Record the unknown numbers and your observations in the attached data table.

Data and Observations: Spectra of Gases

Element: _____

Diagram of Spectrum:

Element: unknown _____

Diagram of Spectrum:

Data and Observations: Flame Test of Known Compounds

Formula	Metal	Color of Flame
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

Unknown # _____

Unknown # _____

Using your results above, identify each unknown (three total, one from the spectra of gases and two from the flame tests). If you aren't certain what unknown you had, you may make a list of possibilities.

Why does the procedure direct you to only identify the metal component of the unknown from the flame tests?

Identification of Drugs and Poisons Lab

Much of the chemical work in a forensic crime lab involves the analysis of drugs. A suspected drug sample will be spot tested to determine if the sample does in fact contain a drug and if so what class of drug is present. Other tests such as TLC or IR may be completed to definitively identify the drug. For many reasons including the drug license requirements and potential dangers of the reagents required to test real drug samples we will be completing a mock version of this experiment using more benign chemicals on a series of known compounds not requiring a drug license. We will use these results to potentially identify an unknown and then confirm our id using IR.

Physical properties including color, appearance, solubility in water and pH may help separate different chemicals into smaller groups. Sodium bicarbonate, NaHCO_3 , tests for the presence of acids. A positive test result is observed with the presence of bubbles. Ferric chloride, FeCl_3 , tests for the presence of phenols. In the presence of phenols, the solution will turn purple. Potassium permanganate, KMnO_4 , is an oxidizer and will test positive for alcohols, aldehydes and ketones. A positive test is indicated by the formation of a brown precipitate (solid). Benedict's test is used to identify a reducing sugar. Reducing sugars are able to form a free aldehyde or ketone in solution. In the Benedict test a brick-red precipitate indicates a positive result. The color of a positive Benedict's test may also appear green, yellow, orange or red.

Clearly, from the descriptions of the positive test results above, we are not testing real drugs, although some of the functional groups present may be in known classes of drugs. The goal of this lab is to give you an idea of how spot tests can be used to determine the general class of a chemical. Once the spot tests have been completed, further tests will be run such as IR to identify what the unknown chemical is.

Procedure

Physical Properties

- 1) Record your observations about the physical properties of each known and your unknown. Include observations about the color, texture and general appearance of each sample. **Do not taste any chemicals in the lab, ever!**

Solubility Tests in Water, pH Determination and Sample Preparation

- 1) Add 5 mL of distilled water to separate, labeled test tubes.
- 2) Add a pea-size quantity of each solid to each labeled test tube.
- 3) Mix gently.
- 4) Record your observations. A soluble solid will dissolve completely or almost completely while an insoluble solid will hardly dissolve at all.
- 5) Measure the pH of each solution by touching drop of the solution to a piece of pH paper. Please note, multiply solutions can be tested on different places of the pH paper.
- 6) Keep each solution for future tests.

Please complete Table 1 on page 51 prior to beginning the color tests.

Table 1: Pre lab	NaHCO ₃ Test	KMnO ₄ Test	FeCl ₃ Test	Benedicts Test
Functional groups testing positive				
Positive test result				

Compound	Solubility, pH	NaHCO ₃ Test	KMnO ₄ Test	FeCl ₃ Test	Benedicts Test
Aspirin:					
Caffeine:					
Sucrose:					
Fructose:					
Ascorbic Acid: (vitamin C)					
Unknown #:					

Restoring Serial Numbers on Metals Lab

Many objects are marked or stamped with a serial number. When an object is marked more than just the surface layer is impacted. The molecules or crystals below the surface of the stamp are under strain compared to the surrounding molecules or crystals. When a chemical reagent is applied it will react faster with one area than another making the stamp or mark visible. The hidden mark may not stay permanently visible after treatment so a camera is needed to record the results. Different reagents are used to restore serial numbers depending on the composition of the material. Serial numbers on wood can be restored using sodium hydroxide, the active component in many drain cleaners. Marks in plastics and hard rubber can be restored using trichloroethylene, an organic substance. Metals provide the greatest number of options for reagent use to visualize hidden marks. Many of the etching materials for metals contain acid with a metallic ion such as copper. When the surface of an object made from iron has been marked and the mark removed, the iron atoms are under stress and therefore are more susceptible to an oxidation-reduction reaction with copper. The copper ions in the solution react with the solid iron atoms to form solid copper and ionic iron. The reaction is $\text{Cu}^{2+}(\text{aq}) + \text{Fe}(\text{s}) \rightarrow \text{Cu}(\text{s}) + \text{Fe}^{2+}(\text{aq})$. This is a reaction similar to those you observed in the Observing Chemical Reaction lab with the aluminum metal reacting with acid and the aluminum metal reaction with copper ions in solution. Oxidation reduction reactions work with lots of different metals, thus providing the forensic scientist more options for restoring hidden marks. A trained forensic scientist has a reasonably good chance of restoring serial numbers from metals, a fair chance of restoring serial numbers from wood and plastic and a poor chance of restoring marks made on leather. One thing to keep in mind no matter what the surface is: restoring serial numbers takes a great deal of patience!

The basic technique for restoring serial numbers includes cleaning the surface, smoothing the surface usually with a fine emery cloth, and applying the reagent. The surface is smoothed to remove deep scratches that may hold excess reagent solution and also to remove the oxidized coating on the metal's surface. If the reagent is going to be poured on the surface a dam is built to protect the remaining surface. Alternatively a swab can be used to apply the reagent only to the desired area. Fresh reagent is applied every 15 to 20 minutes or the surface swabbed at 2 to 3 minute intervals. Patience is required along with a camera. If nothing happens after an hour or so, try a different reagent. In some cases, the hidden marks may only appear once, nothing like that kind of pressure to get it right the first time! The table below lists the different reagents used depending on the type of metal surface. For all solutions requiring acid, the acid is hydrochloric acid, HCl, the same acid used in the Observing Chemical Reactions lab. (2)

Metal	Reagent
Stainless steel	Copper (II) chloride (CuCl_2) with acid
Cast iron	Copper (II) chloride (CuCl_2) with acid
Brass and copper	Iron (III) chloride (FeCl_3) with acid
Aluminum	Iron (III) chloride (FeCl_3) with acid
Lead	Silver nitrate (AgNO_3)
Silver jewelry	Nitric acid (HNO_3)
Tin	Acid

Procedure

- 1) Carefully clean the surface with acetone to dissolve any grease.
- 2) Using an emery cloth or fine sand paper, polish the surface to a shine. Do not use circular marks and continue smoothing the surface until the grinding or filing marks disappear.
- 3) Clean the surface again with acetone and avoid getting fingerprints on the metal's surface.
- 4) Pour a few mL of reagent into a small beaker to use at your lab bench. Dip a cotton swab into the reagent and slowly move the swab across the suspect area.
- 5) Watch the surface closely. If numbers or letters begin to appear, write them down. Some parts of the serial number may disappear as others appear.
- 6) Continue swabbing the suspect area at 2 to 3 minute intervals for at least 30 minutes before stopping.
- 7) Optional: photograph any numbers as they appear.
- 8) Clean up your area.

Observations:

What type of metal surface did you have? List all that apply from the table on the previous page.

What, if any, numbers were you able to recover?

Were you correct? What were the numbers filed off?

Literature Cited

- 1) Saferstein, Richard; *Criminalistics*, 9th ed.; Pearson Prentice Hall: New Jersey, 2007.
- 2) Meloan, Clifton E.; James, Richard E.; Saferstein, Richard; *Criminalistics Lab Manual*, 9th ed.; Pearson Prentice Hall: New Jersey, 2007.

Identification of Unknowns by Infrared Spectroscopy Lab

During the spectroscopy lab, we observed the visible portion of the electromagnetic spectrum. Many other sections of the electromagnetic spectrum can be used to analyze unknown substances. X-rays, ultraviolet rays, visible light and infrared rays can all be used by the forensic scientist. Different chemicals will absorb different types of electromagnetic radiation in different ways. These differences can be used to identify an unknown substance. Organic molecules (those containing carbon) absorb infrared radiation (IR) causing the atoms or groups of atoms in the molecule to vibrate in a characteristic manner.

In an infrared spectrometer, infrared radiation is passed through a sample and compared to a reference beam. A plot of absorption versus frequency or wavelength is generated. The location of an IR absorption band at a certain wavenumber (location) is characteristic for groups of atoms called functional groups. Each molecule has its own characteristic IR spectrum and therefore serves as a fingerprint for the molecule. Because of the uniqueness of IR spectra, this technique can be used to positively identify an unknown. We can move beyond comparing an unknown to a known sample to determine the possibility of a common origin to actually identifying what the unknown is! The IR is a necessity for modern forensic labs.

Procedure:

- 1) Obtain a sample of an unknown.
- 2) Record your unknown number.
- 3) Briefly describe the physical properties of the unknown.
- 4) Obtain an IR spectrum for your unknown following the instructions give by your instructor or located near the IR or summarized below.
 - a. Turn the computer and the IR on. The computer and IR will likely already be turned in and ready to go.
 - b. Open EZ OMNIC.
 - c. Using the buttons towards to top of the screen, click Col Smp (collect sample) and follow the prompts on the screen.
 - d. Enter a name for the spectrum and click OK.
 - e. Click OK in the collect background box after ensuring the sample plate is empty and the lid of the IR is closed.
 - f. After a confirmation box appears, add the unknown sample to the yellow sample plate. Add enough of your unknown to cover the plate but not so much that it overflows and runs into the bottom of the IR. You should be able to see the sample of the metal ring around the plate.
 - g. Close the lid and click OK.
 - h. When the computer asks “add to window?” say yes.
 - i. Check to see if %T appears on the y-axis, if not click on %T (%transmittance).
 - j. Under view, click on display limits. Set the x-axis range to be 4000 to 460 cm^{-1} and the y-axis range to be 0 to 100%.
 - k. Use Save as under File to save the spectra to the student folder on the Science server. Direct the computer to save the file in the G-drive, student, chemistry, Omnic Data, CH 114 folder.
 - l. Clean up as directed by your instructor. Use a Kleenex tissue to absorb the remaining unknown from the sample plate.

- m. Rinse the sample plate with ethanol and wipe clean with a Kleenex.
 - n. Click the clear button at the top of the screen.
- 5) Analyze the spectra on a different computer in the same room as the IR.
- a. Open Omni program.
 - b. Open the saved spectra.
 - c. Print spectra.
 - d. Click on the analyze menu at the top of the screen, go to library set-up.
 - e. Click on the libraries you wish to use to analyze your spectrum. Suggested libraries to use are: CH114SP09 and Georgia State Crime Lab. Highlight the library you want to use, click the add button in the center of the screen.
 - f. Click the search button at the bottom of the screen.
 - g. Record which three spectra most closely match your unknown. Include the name of the compound, the match percentage and the library source of the match.
 - h. Report your results on the board in the lab for others in the class to see.

Questions from Burning Down the House Case Study, please answer these questions on a separate sheet of paper or the back of one of the following pages.

- 1) What is the legal definition of arson?
- 2) What types of evidence have been used to convict individuals of arson? How will you be able to tell if arson has been committed?
- 3) What is the significance of the term “point of origin”?
- 4) Give that the analytes of interest are volatile, what techniques could you use to conduct your investigation?
- 5) Which of the techniques you identified in the previous question do you think will work the best in your investigation? Why?

1) Complete the following table.

Function Group	Absorption Peaks (cm^{-1})	Sample Structure
Alkane	2900-3000	
Aromatic	3000-3200	
Alcohol	3610-3640 and 1050	
Ketone	1715 and 1100	
Aldehyde	1725, 2820, and 2720	
Carboxylic acid	1760, 1710, and 2500-3000	
Ester	1735, two between 1300-1050	

2) What unknown did you have? Briefly explain.

- 3) Draw the structure of your unknown and identify its functional groups.

- 4) Attach the IR spectra of your unknown, and on the spectra identify the functional group of at least two of the peaks.

Burning Down the House Case Study

- 5) Based on your results and those of the class, was the fire described in the Burning Down the House case study the result of arson? Briefly explain.

- 6) Can Dr. Stanforth be ruled out as a potential suspect? Briefly explain.

Literature Cited

1. Saferstein, Richard; *Criminalistics*, 9th ed.; Pearson Prentice Hall: New Jersey, 2007.
2. Meloan, Clifton E.; James, Richard E.; Saferstein, Richard; *Criminalistics Lab Manual*, 9th ed.; Pearson Prentice Hall: New Jersey, 2007.
3. Burning Down the House Case Study, National Center for Case Study Teaching in Science.

Blood Identification and Typing Lab

Blood is a common type of physical evidence found at crime scenes. Many substances may appear to be blood. Before time and energy are devoted to determining if the blood is human or not and before work begins to identify other blood related factors, such as blood type, the forensic scientist will use a presumptive test to first determine if the sample is blood. Once the sample is presumptively identified as blood, the origin of the blood as well as blood type can be determined. If necessary, DNA can be collected and analyzed from the blood samples as well. Although visual characteristics and a positive presumptive test are good indicators of a sample being blood, presumptive tests do not confirm that a sample is blood; they only indicate the likeliness of the sample being blood. Only a positive blood crystal test, the presence of human protein or successful DNA amplification will confirm and positively identify a sample as blood (3).

Different methods are available for use as preliminary color tests, all of which are presumptive tests. Hematest tablets, the Kastle-Meyer color test and luminol are three examples. Use of Hematest tablets has been replaced by the Kastle-Meyer color test. The Kastle-Meyer test is more sensitive and more specific for blood. In this test, a solution of reduced phenolphthalein turns bright pink in the presence of blood or other oxidizers. The test works because of the presence of an enzyme in blood that is part of the peroxidase group of enzymes. Peroxidases accelerate the oxidation of certain organic compounds by peroxide. Enzymes are catalysts; they speed up reactions but are not consumed by the reaction. Because peroxidases are oxidizers, other oxidizing agents will also test positive, in other words, the background presence of oxidizers such as bleach or other cleaners may cause a positive presumptive test result for blood. If this is the case, a substrate control is tested. In a substrate control, an area on the same surface as the targeted stain is collected. Negative controls are also tested (or blanks). One note of caution with the Kastle-Meyer- color test, over time even a negative control will turn pink (test positive) so observations should be made in a reasonable amount of time.

Luminol is also used to presumptively test for blood. In this case, the reaction of luminol with the iron in blood produces light, instead of a color change. Luminol is also more sensitive than the Kastle-Meyer color test and can detect bloodstains diluted up to 300 000 times. Because the reaction of luminol with blood produces light, the surrounding area must be dark. Luminol can be sprayed over a large area and the area observed in the dark. As an added benefit, luminol does not interfere with DNA testing of suspected blood samples.

In this lab we will use both the Kastle-Meyer color test and luminol to presumptively test for blood. We will also observe how the reagents react with other blood-like samples, including some oxidizers (giving us false positives). Some of the samples used in this lab are in fact real blood from an animal source, however the samples have been ordered from a science supply company. The provided samples are free from contaminants but should still be handled with care. Please wear gloves. Due to safety concerns, we are not able to type our own blood.

Once the presumptive tests are complete and if they indicate the likely presence of blood, other tests as described above must be completed to confirm whether the sample is in fact blood. Assuming that the sample is blood, specific factors in the blood may be useful for the forensic scientist to identify who the blood belongs to. Previously, blood typing was used. Blood typing consumes evidence and produces less strong conclusions than DNA analysis and thus blood typing has been replaced with DNA analysis. When blood typing was used, it along with the analysis of several other factors were combined to give a frequency of occurrence. The forensic

scientist would conclude there was a one in 20000 chance the suspect's blood matched the collected sample. Although the odds were less than are possible with DNA testing, many innocent people would be cleared and the guilt of the suspect corroborated.

Blood type was discovered in 1900 by Karl Landsteiner (1). He observed that blood of one person would not mix with the blood of another person but instead would clump or agglutinate. Landsteiner identified four blood types, A, B, AB and O. Approximately 43% of the population has O type blood, while 42% has type A, 12% type B and 3% type AB. As early as 1916, blood typing was used in courts of law. Other factors have also been determined and used to identify or characterize blood samples. One other common factor is the Rh factor. Common blood tests during pregnancy test for this factor. In this lab we will test blood samples only for A, B, O or AB type.

Procedure

Presumptive Tests for Blood: Kastle-Meyer (2)

Note: when applying a drop of any reagent to the swab to prevent contamination, please do not touch the dropper to the swab.

- 1) Place two swabs in a test tube holder, approximately three inches apart (far enough apart so they don't touch each other).
- 2) Apply one drop of known blood to swab #1, this is the positive control.
- 3) Apply one drop of water to swab #2, this is the negative control.
- 4) Apply one drop of hydrogen peroxide to each swab.
- 5) Apply one drop of phenolphthalein solution to each swab.
- 6) Record your observations.
- 7) Set aside and observe once more before leaving lab.
- 8) Test each piece of evidence by adding a drop of water to two new swabs. Set one of the swabs aside as the negative control.
- 9) Roll the other swab over the evidence stain.
- 10) Apply a drop of hydrogen peroxide to each swab, then a drop of phenolphthalein and record your observations.
- 11) Be sure to clearly label each piece of evidence and handle carefully to prevent contamination.

Luminol Test

- 1) Place a drop of blood on a small watch glass.
- 2) Spray the sample with luminol and observe in the dark.
- 3) Try wiping up a known blood sample and spraying with luminol. Record your observations.
- 4) Test other substances with luminol and record your observations.

Procedure for Blood-Typing: follow the instructions provided with the kits. Record your observations. A diagram may be helpful.

Observations: In the space below generate a data table listing the results for each test your performed. Be sure to clearly indicate which sample(s) you tested and the results for any unknowns.

Questions:

- 1) Do you think the ABO blood typing could be done on a smaller sample of blood? What difficulties do you foresee?

- 2) How do you suppose a dried blood stain would be typed?

Literature Cited

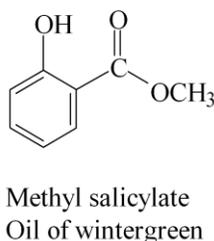
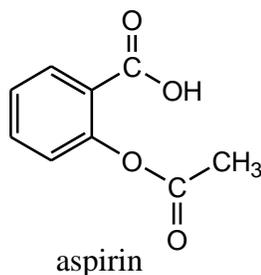
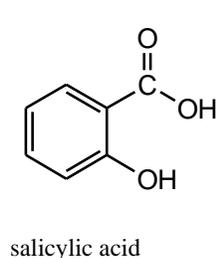
- 1) Saferstein, Richard; *Criminalistics*, 9th ed.; Pearson Prentice Hall: New Jersey, 2007.
- 2) Meloen, Clifton E.; James, Richard E.; Saferstein, Richard; *Criminalistics Lab Manual*, 9th ed.; Pearson Prentice Hall: New Jersey, 2007.
- 3) Personal email communication with Jennifer Riedel, Forensic Scientist, Oregon State Crime Lab.

Visible Spectroscopy Lab

We have seen the use of infrared radiation to characterize an unknown substance. Ultraviolet and visible spectroscopies also play an important role in the forensics lab, although for different reasons. The spectrum produced from ultraviolet radiation is much simpler (contains fewer peaks) than an IR spectrum and therefore is not as helpful for identifying an unknown substance. UV spectroscopy is therefore useful for making a probable identity. Visible spectroscopy is not used to identify an unknown substance but can be used to determine the unknown concentration of a substance, making it a useful quantification technique. Both UV and visible spectroscopy measure the amount of light absorbed by a solution. The amount of light absorbed (or transmitted) is directly related to the concentration of the substance. Each substance has characteristic wavelengths of maximum absorbance. By determining the maximum wavelength of absorbance, the concentration of one compound in a mixture can be determined using Beer's law.

Beer's law states the absorption of light (A) is equal to a constant (k) times the concentration of absorbing material (c): $A = kc$. The amount of light absorbed is directly related to the concentration of the absorbing substance. The greater the concentration of the absorbing substance, the more light is absorbed by the substance (or less light is transmitted). By plotting the absorbance versus concentration of known solutions a calibration curve can be generated and then used to determine an unknown concentration of the same absorbing substance. Remember that when using visible spectroscopy, the forensic scientist knows what the absorbing substance is but not how much of that substance is present in the solution. The amount of substance present is the concentration. Concentration has many units, such as percent by volume or mass, mass per volume (same units as density but a different property) and the chemist's favorite unit molarity (moles per liter). For this lab, the units of concentration will be milligrams per 100 milliliters (mg/100 mL) or milligrams per deciliter (mg/dL). Either unit will work because 100 mL = 1 dL.

Visible spectroscopy may be used by the forensic chemist to determine the concentration of drugs (both legal and illegal) in a collected sample. A group of chemicals called salicylates are found in many over the counter analgesics (painkillers) containing aspirin. Oil of wintergreen is also a common source. Salicylates are all derived from salicylic acid.



Taking in large doses (by overdose) of aspirin or ingestion of large amounts of oil of wintergreen, may lead to salicylate poisoning. Symptoms may include hyperventilating or other breathing problems, dehydration, confusion, nausea, dizziness as well as more serious symptoms associated with metabolic acidosis. (3)

Salicylates will react with iron salts to produce a violet color. The brightness of the purple color is proportional to the concentration of salicylate in the solution. A brighter purple

color is produced from a more concentrated salicylate solution (brighter purple = more salicylates). The violet color absorbs at a wavelength of 540 nm. The observation of a purple color when an iron salt is added to a blood serum sample quickly identifies the presence of salicylates in severe poisoning cases.

In this lab, we will use visible spectroscopy to generate a calibration curve to determine the concentration of salicylates in an unknown sample. In the forensic lab, this experiment would be completed using a blood serum sample; however for safety reasons we will reproduce the experiment without the use of blood. We're also going to use a Food Dye, Allura Red instead of salicylates. You will observe a red color instead of purple.

Procedure:

Preparation Solutions and Generation of Calibration Curve

- 1) In small test tubes, carefully measure and mix the following:

Cuvette	Standard	Distilled water (mL)
1 (blank)	0.00 mL	4.00
2	0.20 mL	3.80
3	0.60 mL	3.40
4	1.00 mL	3.00
5	1.40 mL	2.60
6	2.00 mL	2.00

The standard is 5.0 mg/100 mL Allura Red. Be sure to use distilled water. The total volume for each solution is 4.00 mL.

Calibration of the spectrophotometer and Determination of λ_{max} , the wavelength of maximum absorbance.

- 1) Start the Logger *Pro* 3.4.5 software, a rainbow should appear on the screen.
- 2) To calibrate the Spectrometer, select Experiment > Calibrate > Spectrometer. The calibration dialog box will display the message: "Waiting ... seconds for lamp to warm up." Allow the spectrometer to warm up for at least three minutes. Follow the instructions in the dialog box to complete the calibration. The small container used to hold the sample is referred to as a "cuvet". When handling the cuvet, touch the ridged sides only to avoid getting fingerprints on the windows. Fill the cuvet $\frac{3}{4}$ full the blank listed in the table above (cuvet 1). Insert the cuvet in the spectrometer with the clear side of the cuvet lined up to the arrow and lightbulb on the spectrophotometer. Click OK.
- 3) Return the solution to the original test tube (just in case you need to use it again).
- 4) Fill another cuvet $\frac{3}{4}$ full with solution 6, click the green Collect button.
- 5) Click Stop (red button) to end data collection.
- 6) Return the solution to test tube or store in the cuvet.
- 7) Does your graph look like a bell curve? If not, repeat steps 5 to 7. If that doesn't generate a bell curve please ask your instructor for assistance.
- 8) Under experiment, select store latest run.
- 9) Repeat steps 4 to 8 with remaining solutions.

- 10) To optimize the view of the absorbance spectrum that you have just collected, select Analyze> Autoscale > Autoscale.
- 11) To find the wavelength of maximum absorbance (λ_{max}) select Analyze, Examine. This will bring up a line on the screen that you can move to the wavelength of maximum absorbance. Record this value.
- 12) Record the absorbance of each solution at the same wavelength (λ_{max}).

For all solutions, record the absorbance at λ_{max} .

Determination of Unknown

- 1) Measure and record the absorbance as described above.
- 2) Print the absorbance spectrum by typing Apple-P, or by going to the file menu and selecting Print Graph. Your printed graph should show the absorbance of all solutions.

Calculations: In order to generate a calibration curve, the absorbance will be plotted on the y axis and the known concentration of salicylate on the x axis. To calculate the known concentration of Allura Red, we need to consider the dilution of the standard by the addition of water. Each solution has a total volume of 4.0 mL.

To calculate the concentration of Allura Red in the solution we need to multiply the standard concentration (5.0 mg/100 mL) by the volume of standard used in each cuvette. This gives us the total milligrams of Allura Red. To find the concentration, we need to divide the total milligrams of Allura Red by the total volume of the solution (4.0 mL). An example follows for cuvet 2:

$$\frac{5.0 \text{ mg}}{100 \text{ mL}} * 0.20 \text{ mL} = 0.010 \text{ mg Allura Red}$$

then
$$\frac{0.010 \text{ mg Allura Red}}{4.0 \text{ mL}} = 0.0025 \text{ mg/mL}$$

In the space below, show one sample calculation for the known concentrations of the solutions used to generate the calibration curve.

Observations and Data:

Observations:

$\lambda_{\max} =$ _____

Cuvette	Absorbance at λ_{\max}	Calculated Concentration
1 (blank)		0.00 mg /mL
2		0.0025 mg/mL
3		
4		
5		
6		
Unknown		

Using Graphical Analysis or another graphing program on your computer or calculator, graph the concentration of each solution on the x axis and the absorbance at λ_{\max} on the y axis. This is the calibration curve. Determine the slope of the (hopefully) straight line. To do this click the R= button towards the top of the screen in Graphical Analysis.

From the calibration curve, determine the concentration of salicylate in the unknown. Briefly explain how you determined the unknown concentration. Be sure to include a copy of your calibration curve with your lab. Using the equation of the straight line you may calculate the concentration of the unknown (x) by substituting the measured absorbance in for y in the equation $y = mx+b$ or you may use the live mouse in Graphical Analysis.

Isolation of DNA Lab

DNA, deoxyribonucleic acid, carries our genetic information. DNA will confirm the presence of blood and can identify one person out of ten billion. With only six billion people on the planet, DNA can uniquely identify each of us. Many advances have been made in the use of DNA in forensic science and many more are continuing to be made. During the late 1980's and early 1990's restriction fragment length polymorphisms (RFLP) were used to type DNA. By the mid 1990's the polymerase chain reaction (PCR) replaced RFLP as the dominant method for typing DNA. Short-tandem repeats (STRs) are now the latest and best method for DNA typing (1). What's next?

In this lab we will isolate DNA from wheat germ and a fruit or vegetable and then we will analyze a prepared sample of DNA using gel electrophoresis. Unfortunately, we do not have the reagents or equipment necessary to replicate our isolated DNA samples, although for a forensic scientist this would be a key aspect their work.

In order to extract DNA from a cell's nucleus, the cell membrane must be broken apart and the DNA coiled around the histones released. The DNA must then be separated from the histones by denaturing the proteins making up the histones. The denaturation of the proteins also deactivates the enzymes that catalyze reactions breaking DNA into smaller fragments. In this experiment, dishwashing detergent will be used to break the cell and nucleus membranes by emulsify the fats of the membranes and breaking them down. The proteins making up the histones will be denatured using heat and an enzyme found in meat tenderizer. DNA can be isolated with or without using a salt solution. A salt solution works because the negatively charged phosphate groups make DNA soluble in a salt-water solution. Nonpolar isopropyl alcohol is then used to precipitate the DNA from the solution.

Procedure

Using Heat to Denature Proteins, The Effect of Detergents on the Cell Membrane and Using an Enzyme to Denature Interfering Proteins

- 1) You will need approximately 1.5 grams of wheat germ total, split into three beakers each containing 0.5 grams.
- 2) Add 150-mL of distilled water to a 250-mL beaker and heat to at least 55°C but no warmer than 60°C. Stir the water periodically.
- 3) Using a graduated cylinder, add 10-mL of heated water to two of the wheat germ samples.
- 4) Add 10-mL of room temperature water to the third wheat germ sample.
- 5) Swirl the mixture constantly for 3 minutes.
- 6) Add 10-12 drops of liquid dishwashing soap and swirl constantly for an additional 5 minutes to one of the wheat germ samples with heated water and to the sample with room temperature water.
- 7) To the room temperature beaker, add 0.1 grams of meat tenderizer. Swirl constantly for 3 minutes.
- 8) Let the beakers sit undisturbed for 1-3 minutes to allow the solid material to settle.

- 9) Using a clean plastic pipet remove enough of the cloudy liquid top layer to fill a test-tube 1/3 full. Do this carefully to avoid removing settled solid. Tilt the test tube and use a plastic pipet to remove any foam or bubbles.
- 10) Measure 4-mL of alcohol in a graduated cylinder.
- 11) Tilt the test tube with the cloudy liquid and slowly pour the alcohol down the inside of the test tube so the alcohol forms a layer on top of the cloudy liquid. Do not mix the two layers together.
- 12) The released DNA from the wheat germ is in the lower, water containing level. You may have noticed some DNA precipitate as a filmy, white solid at the boundary between the two layers. To precipitate more DNA from the water layer carefully insert a wooden splint (or use a pipet) through the alcohol layer to the bottom of the test tube. Slowly pull the splint or pipet upwards. As the DNA containing water layer comes in contact with the alcohol, the DNA will precipitate and form a stringy film. If this step is done repeatedly a significant amount of DNA can be formed and observed in the alcohol layer.
- 13) Let the test tube sit for 10-15 minutes. Precipitated DNA will float to the top of the alcohol layer.
- 14) Record your observations.

Extracting DNA from fruit or vegetables using salt water.

- 1) Prepare an ice bath by filling a 400-mL beaker about ¼ full of ice and then adding 200-mL of water.
- 2) In an Erlenmeyer flask, mix 1.5 grams of sodium chloride (salt), 5.0 grams of sodium bicarbonate (baking soda), 5 mL laundry detergent and 120 mL distilled water.
- 3) Chill the solution in the ice bath.
- 4) Obtain 10-mL of blended fruit or vegetable sample in a 50-mL beaker.
- 5) Add approximately 20 mL of chilled salt/detergent solution and stir vigorously for at least 2 minutes.
- 6) Cool 10-mL isopropyl alcohol in the ice bath.
- 7) With a pipet, slowly and gently add 10 mL of chilled alcohol solution to the DNA solution so the alcohol forms a top layer. Let the alcohol run down the side of the cylinder so two distinct layers form.
- 8) Using the tip of the pipet gently stir the DNA solution just below the alcohol layer, gently lifting the solution toward the alcohol layer.
- 9) Look for cloudiness and the formation of DNA strands.
- 10) Record your observations.

Replication or DNA by PCR and Analysis of DNA by gel electrophoresis: please follow the instructions provided to in the handout from class. Record your observations by sketching the developed gel or taking a photo. If your gel does not develop, please ask the instructor for a copy of a “developed” gel.

Record your observations for each extracting method:

Using Heat to Denature Proteins

The Effect of Detergents on the Cell Membrane

Using an Enzyme to Denature Interfering Proteins

Extracting DNA from fruit or vegetables using salt water. What fruit or vegetable was used?

Analysis of DNA: PCR and gel electrophoresis

Adopted from Carolina® Student Guide

DNA, deoxyribonucleic acid, carries our genetic information. DNA will confirm the presence of blood and can identify one person out of ten billion. With only six billion people on the planet, DNA can uniquely identify each of us. Many advances have been made in the use of DNA in forensic science and many more are continuing to be made. During the late 1980's and early 1990's restriction fragment length polymorphisms (RFLP) and gel electrophoresis were used to type DNA. RFLP DNA typing was the first scientifically accepted protocol in the United States used for the forensic characterization of DNA. By the mid 1990's the polymerase chain reaction (PCR) replaced RFLP as the dominant method for typing DNA. PCR required shorter strands of DNA than RFLP could provide. Short-tandem repeats (STRs) and PCR are now the latest and best method for DNA typing (1). Gel electrophoresis has now evolved to capillary electrophoresis to separate the DNA fragments. What's next?

In order to analyze a sample of DNA, the DNA must first be collected, then isolated. Only a small amount of DNA will be present in the isolated sample, so copies of the DNA must be made using the polymerase chain reaction, and then finally the DNA can be analyzed.

In this lab we will isolate DNA from wheat germ and a fruit or vegetable and we will use PCR to replicate a different sample of DNA and then analyze the DNA using gel electrophoresis. Unfortunately, we do not have the reagents or equipment necessary to replicate our isolated DNA samples, although for a forensic scientist this would be a key aspect their work.

In order to extract DNA from a cell's nucleus, the cell membrane must be broken apart and the DNA coiled around the histones released. The DNA must then be separated from the histones by denaturing the proteins making up the histones. The denaturation of the proteins also deactivates the enzymes that catalyze reactions breaking DNA into smaller fragments. In this experiment, dishwashing detergent will be used to break the cell and nucleus membranes by emulsify the fats of the membranes and breaking them down. The proteins making up the histones will be denatured using heat and an enzyme found in meat tenderizer. DNA can be isolated with or without using a salt solution. A salt solution works because the negatively charged phosphate groups make DNA soluble in a salt-water solution. Nonpolar isopropyl alcohol is then used to precipitate the DNA from the solution.

In order to analyze a DNA sample, it is first collected from an individual. Samples from skin, hair, saliva, blood, or other bodily fluids will work. The DNA sequence collected from all the cells in the body is identical regardless of the cell type. The amount of DNA isolated from a forensic sample, and extracted by means similar to what's described above, is generally not large enough to analyze directly. Copies of the DNA to be analyzed need to be made.

The polymerase chain reaction (PCR) generates large quantities of a specific DNA sequence from a very small amount of starting DNA isolated from skin, or hair, or saliva or another bodily fluid. The method leads to exponential amplification of the DNA region by

doubling the number of copies after each cycle. Thirty five cycles from two originals leads to 34, 359, 738, 368 copies of DNA, that's 2^{35} or over 34 billion copies.

A PCR reaction requires template DNA, free nucleotides, primers, DNA polymerase, water and salts for buffering. Four steps are necessary:

- 1) Denaturation: heat the DNA to near boiling so the two strands of DNA will separate.
- 2) Annealing: Complimentary primers bind to the complementary sequences after lowering the temperature to 55-72°C depending on the primer. DNA primers are 18 to 22 bases long and are synthesized in a lab. One primer binds to each DNA strand and is the reverse complement of the strand. The region between the two primers is the sequence of DNA to be amplified.
- 3) Extension: DNA synthesis occurs as Taq polymerase adds nucleotides to the 3' end of the primer. DNA is copied in the 5' to 3' direction. During this step, the two primers produce products that overlap each other. Taq polymerase is an enzyme that catalyzes the DNA synthesis reaction. The Taq polymerase used in PCR comes from bacteria living in very hot environments such as thermal vents or hot springs. The bacteria have evolved to produce proteins resistant to denaturation when heated.
- 4) Repeat: Once synthesis is complete, the DNA is heated to separate the strands. The reaction is cooled so the primers can anneal and the DNA synthesis can occur. The second round of synthesis occurs not only on the original strands but also on the copied strands with each cycle doubling the original number of strands.

Once the DNA copies have been made, the short-tandem repeats need to be analyzed by electrophoresis. The short-tandem repeats are different sizes so separating the fragments based on size will create a DNA fingerprint. Each DNA sample will be pipetted into a small well in an agarose gel. An electric current is then passed through the gel and the DNA migrates towards the positive electrode because of the negatively charged phosphate groups in the DNA backbone. The smaller DNA fragments pass through the gel more quickly than the larger fragments. Once the current is stopped, the bands will remain in the gel. Staining may be necessary to make the DNA bands easier to see.

The PCR products generated from each sample are compared with one another and checked for matching patterns. The approximate size of each band can be determined by comparing the distance the band traveled with the distance travelled by a DNA marker.

The Case: DNA samples from the victim, crime scene, and two suspects have been submitted to the lab for analysis. The DNA has already been isolated from the cells. Your job is to replicate the DNA with PCR and analyze the fragments with gel electrophoresis. To limit personal bias influencing the results, the samples will not be labeled with their origins. A DNA ladder with known sizes of DNA will be used to estimate the size of the fragments. Two additional samples will serve as controls. A sample of genomic DNA from a coworker will be a positive control; this sample is known to work with the protocol. The lack of bands in the positive control indicates a problem with the PCR reaction. Water will be used as a negative control. If DNA fragments are observed in the negative control, contamination likely occurred, and may provide false results.

Procedure

Sample preparation

- 1) Use a waterproof marker to label seven PCR reaction tubes with numbers 1 to 7 (= Tube #). Label each side of each tube.
- 2) Carefully open the tubes, making sure the bead remains inside.
- 3) To avoid contamination:
 - Do not touch any of the solutions
 - Do not touch the inside of the tubes
 - Do not breathe on the samples

Very small volumes of all samples are required for this lab. Each group running a PCR will need to designate one person to ensure all components are added to each sample reaction tube. Other students in the lab will be assigned one sample to deliver to each group's labeled reaction tubes.

Eight students are needed to deliver the reagents: primer mix, samples 1 to 6, and sterile water. Each tube will contain one bead. The bead contains Taq, buffer and free nucleotides.

	Sample 1	Sample 2	Sample 3	Saliva Sample	Skin Sample	Negative Control	Positive Control
Tube #	1	2	3	4	5	6	7
Primer Mix	20 μ L	20 μ L					
DNA Sample	5 μ L Sample 1	5 μ L Sample 2	5 μ L Sample 3	5 μ L Sample 4	5 μ L Sample 5	5 μ L sterile water	5 μ L Sample 6

Please pay attention to the Tube # and the Sample #. For Tubes # 1 to 5, the sample number is the same. However, Tube #6 is the negative control so it has no template DNA and instead contains water. Tube # 7 is the positive control and contains DNA Sample #6.

- 4) All quantities are delivered with a micropipette. Be sure to use a new pipet tip for each different sample. A new pipet tip is not needed to deliver the same sample to different reaction tubes.
- 5) Make sure each tube is securely sealed and placed into the preprogrammed thermocycler.

Program the PCR:

Initial denaturing: 93°C for 2 minutes.

Step 1: 93°C for 30 seconds for continued denaturing

Step 2: 60°C for 30 seconds for annealing

Extension: set by PCR program

Repeat steps 1 and 2 for a total of 25 times

Hold at 4°C

Gel electrophoresis

Agar gels have been prepared.

- 1) Carefully remove the comb from the gel. Please return the comb to the supply cart; do not throw it away.
- 2) Remove the tape from each end of the gel.
- 3) Place the gel carefully in the electrophoresis chamber, being sure to align the electrodes; black end to black end, red end to red end.
- 4) Fill the electrophoresis chamber with buffer so the buffer just covers the gels. The sample wells must be completely submerged.
- 5) Load each sample into a well following the table below. If you happen to change the order of the wells, please make a note of the difference.

Well #	1	2	3	4	5	6	7	8
Sample	Sample 1	Sample 2	Sample 3	Saliva	Skin	Water	Known DNA	DNA ladder

To load the wells:

- a) Slowly draw the contents into the pipet.
 - b) Use two hands to steady the pipet over the well.
 - c) Dip the pipet tip through the surface of the buffer and slowly release the sample in the well. Be careful to not puncture the bottom of the well.
- 6) If the electrophoresis chamber will have a second gel, load the gel into the chamber, cover with buffer and load each sample as described above.
 - 7) Cover the electrophoresis chamber with the electrodes lined up correctly (red to red, black to black).
 - 8) Turn on the power supply, set to high and watch! Make sure you can see tiny bubbles coming from the wires in the chamber. If you have plugged in and turned on the chamber and don't see bubbles, notify your instructor.
 - 9) Run the gel until the loading dye has moved about 5 cm from the well.
 - 10) Turn off the power and carefully remove the gel from the chamber. Be sure the developed gel doesn't slip out of the mold and fall to the floor.

3) If the two suspects in the gel portion of the lab had been identical twins, how would the results differ?

4) If more than two rows of bands look alike in the gel, what might have happened during gel loading?

CH 114 Learning Objectives, Chapter Summaries and Study Guide for Exam #1

Introduction to Forensic Chemistry; after completing this section you should be able to:

Define and distinguish forensic science and criminalistics

Describe the services of a typical comprehensive crime lab as well as other specialized services available to law enforcement personnel

Compare and contrast the Frye and Daubert decisions relating to the admissibility of scientific evidence in the courtroom

Explain the roles and responsibilities of the expert witness

Define and explain the importance of Locard's exchange principle to forensics

Physical Properties: Glass and Soil; after completing this section you should be able to:

Define and distinguish the physical and chemical properties of matter

Use the basic units of the metric system including prefixes to convert between units of measurement and measurement systems

Define and understand the properties of density and refractive index and their use in forensics

Do density calculations

Describe forensic methods for comparing glass fragments

Compare and contrast radial and concentric glass fractures

Determine the direction of impact for a projectile by examining glass fractures; 3R rule

Describe the proper collection of glass evidence

Organic Analysis; after completing this section you should be able to:

Define and distinguish between elements and compounds

Explain how solids, liquids and gases are alike and how they are different

Distinguish between organic and inorganic compounds

Explain the difference between qualitative and quantitative analysis and give examples from lab

Describe and explain the process of chromatography

Explain the differences between thin-layer chromatography, gas chromatography and electrophoresis

Describe the differences between the wave and particle theories of light

Describe the electromagnetic spectrum

Explain how are ultraviolet and infrared spectroscopy are used to identify organic compounds

Explain how mass-spectroscopy is used for identification analysis

Inorganic Analysis; after completing this section you should be able to:

Describe the usefulness of trace elements in the forensic analysis of physical evidence

Explain how a continuous emission spectrum differs from a line spectrum

Understand atomic structure including what are protons, neutrons and electrons, where each is found, and the significance of each particle

Explain how atomic number and atomic mass number differ from each other

Use a periodic table to determine atomic number and identify elements

Write nuclide symbols

Identify metals, nonmetals, alkali metals, alkaline earth metals, transition metal, halogen and noble gases on a periodic table

Explain how an atom absorbs and releases energy in the form of light

Define and identify isotopes

Describe what makes an element radioactive

Explain the ICP process and applications of ICP in forensics

CH 114 Learning Objectives, Chapter Summaries and Study Guide for Exam #2

Forensic Serology; after completing this section you should be able to:

- 1) **List A-B-O antigens and antibodies found in each blood type, A, B, AB and O**
- 2) Describe how whole blood is typed
- 3) **Describe the forensic tests used to characterize blood**
- 4) Describe how are chromosomes and genes different
- 5) **Use a Punnett square to determine the genotypes and phenotypes of offspring**
- 6) **Describe the proper collection and preservation of suspect blood stains**

DNA; after completing this section you should be able to:

- 1) **Describe the structure of DNA including the components of the backbone, the base and complementary base pairing.**
- 2) Understand and explain how base pairing contributes to the double helix structure of DNA
- 3) **Describe the processes of replication, transcription and translation**
- 4) **Given an original strand of DNA, provide the sequence of the template strand, mRNA and amino acid sequence for the protein**
- 5) **Contrast DNA strands coding for the production of proteins with strands containing repeating base sequences**
- 6) **Explain the technology of PCR and how it applies to forensic DNA typing**
- 7) **Understand and explain the structure and use of STRs**
- 8) **Type DNA using gel electrophoresis and capillary electrophoresis**
- 9) Contrast nuclear and mitochondrial DNA
- 10) Explain the use of computerized DNA databases used in criminal investigations
- 11) **Describe what procedures must be followed for the proper preservation of bloodstained evidence for DNA analysis**

Arson; after completing this section you should be able to:

Describe the role the criminalist takes in arson investigations

Describe the goal of searching a fire scene and explain why a warrant is not required

Recognize the telltale signs of an accelerant-initiated fire

Describe how to collect physical evidence at the scene of a suspected arson

Describe what laboratory procedures are used to detect and identify hydrocarbon residues

Describe in general, the chemistry of fire and the types of substances are required and produced

Explain why fire is described as a chain reaction

Explain how non-gaseous fuels burn

List the three basic ingredients of a flaming fire