Curriculum for Investigative Science

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Research Experience for Teachers 2005
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Serology Module Rational / Lesson Plans

I. Rationale

Blood is among the most common forms of evidence found at scenes of violent and brutal crimes. All suspected blood—liquid or dried, animal or human—present suggests a relationship to the offense or persons involved in a crime. Blood may be found in trace amounts, puddles, spatters, smears, or droplets. Blood samples may be collected from suspects and victims for examination and comparison. Blood evidence can help narrow a group of suspects, support identification of a suspect, and even guide the reconstruction of a crime.

The term serology is used to describe a broad scope of laboratory tests that use specific antigen and serum antibody reactions. We devised this module to reflect several areas investigated in forensic serology. These areas answer the most fundamental questions that laboratory examination of blood evidence can answer: (1) Is the substance blood? (2) Is the substance human blood? and (3) If it is human blood, to what blood group does it belong? This unit of study also includes a brief investigation of bloodstain spatter.

We have designed these lesson plans to be easily modified as needed by the teacher. It is our goal that teachers can decide to present all the material in one comprehensive package or just pull out specific activities/topics to use as mini-lessons. In all of the activities, additional suggestions and time saving tips are included at the end.

II. Objectives

A. Cognitive objectives:
Upon completion of this module, the student will be able to:

- Understand terminology associated with serology.
- Explain the historical aspects of serology in forensic science.
- Identify the fundamental biological concepts of the nature of blood.
- Distinguish the information a forensic scientist could gain from blood and blood spatter.
- Identify procedures a forensic scientist would utilize to gain insight about a blood sample.
- Describe the scientific concepts behind blood detection and identify methods used to detect blood (luminol, Kastle-Meyer, fluorescein, and benzidine).
- Recognize methods used to determine if a sample is human blood.
- Comprehend the A-B-O and Rh system of blood typing.
- Explain the significance of blood spatter and the information gained from a blood stain.

B. Skills and Performance objectives:
Upon completion of this module, the student will be able to:

- Identify substances as "blood" based on laboratory observations.
- Utilize laboratory techniques
- Demonstrate proper safety practices.
- Identify various blood types.
- Observe the effect of height on spatter patterns.
- Explore the relationship between angle of impact and pattern formed
- Utilize graphing techniques to analyze data.
- Work cooperatively in a group setting.
III. Materials—see lab activities for detailed descriptions of materials and equipment

IV. Instructional Procedure—based on 85 minute class periods
A. Lesson: Serology Overview
   • Opening
     1) Review Punnett squares
   • Middle
     1) Presentation of terminology, history, and nature of blood (blood agglutination transparency)
     2) Activity: Paternity Suit (criminalistics case form)
   • Closing
     1) Students discuss and defend results from activity

B. Lesson: “Is it Blood?”
   • Opening
     1) Ask “how do forensics scientists detect the presence of blood?” (as seen on CSI or other forensic TV shows) and discuss
   • Middle
     1) Presentation of methods forensic scientists use to detect blood.
     2) Activity: Is it Blood? lab
   • Closing
     1) Discuss results of activity

C. Lesson: “Is it Human Blood?” & “If it is human blood, to what blood group does it belong?”
   • Opening
     1) Ask “once criminal investigators have detected the presence of blood at a crime scene, what is the next step in the process?” and discuss
   • Middle
     1) Presentation of methods to test for human blood
     2) Presentation of A-B-O blood system and Rh factor
     3) Activity: A-B-O Blood Typing Simulation lab
   • Closing
     1) Take and disseminate class data
     2) Analyze and discuss results

D. Lesson: Blood Spatter
   • Opening
     1) Ask “what information do forensic scientist gain from blood stain analysis?” and discuss
   • Middle
     1) Presentation of what bloodstains can reveal (blood stain/spatter transparency)
     2) Activity: Blood Spatter lab
   • Closing
     1) Using the Blood Spatter Activity, have the class pool their data to create an average for each ratio. Make a class graph and compare to group graphs.
     2) Questions: What effect does height have on the ratio? What effect does angle have on the ratio?
V. Activities—see lab activities and teacher materials for detailed information

A. Synthetic Blood
   • Used in nearly all serology module lab activities
   • Can be used simply as a recipe for an excellent blood supplement
   • Can be used as a lab activity for students to reinforce lab techniques

B. Paternity Suit (criminalistics case report form)
   • Paper or dry lab requiring no chemicals
   • Assumed that students have background knowledge of Punnett squares
   • Students use Punnett squares to determine the likelihood of an illegitimate child in hypothetical paternity cases

C. Is it Blood?
   • Use luminol detection method
   • Students investigate a variety of red substances to predict the presence of “blood”

D. A-B-O Blood Typing Simulation
   • Students will determine the blood type of unknown samples
   • Uses milk, vinegar, water, and synthetic blood to simulate blood typing
   • Students pool data to create a percentage and bar graph that reflects the general population’s blood type

E. Blood Spatter
   • Students utilize graphing techniques to determine relationships between height, angle of impact, and drop dimension ratios
## Serology at a Glance

<table>
<thead>
<tr>
<th>Lesson</th>
<th>Students will:</th>
<th>Duration</th>
</tr>
</thead>
</table>
| **Overview**  | 1) review Punnett squares  
                learn serology terminology, serology history, and the nature of blood  
                blood types using Punnett squares (lab) | 1) 15 minutes  
                2) 30 minutes  
                3) 40 minutes |
| **List**      | 1) learn methods used to detect blood  
                explore methods to detect blood (lab) | 1) 20-30 minutes  
                2) 40-60 minutes | 2) |
| **What**      | 1) learn methods to detect human blood  
                explore blood systems and factors  
                investigate A-B-O blood system (lab) | 1) 10 minutes  
                2) 20 minutes  
                3) 20-30 minutes | 3) |
| **Blood**     | 1) learn about bloodstains and blood spatter  
                explore blood spatter patterns (lab) | 1) 20 minutes  
                2) 60-70 minutes |
I. **Overview**

A. **History**
   1. Before 1900s—physicians had attempted to transfuse blood
      - Resulted in coagulation causing instantaneous death
   2. 1901—Karl Landsteiner—discovered blood typing
      - Recognized that all human blood was not the same; distinguishable by its group or type
      - Won Noble Prize in 1930
   3. 1930s—the Rh factor was recognized
   4. 1990s—the advent of DNA technology dramatically altered the approach forensic scientists take toward the individualization of bloodstains and other biological evidence

B. **Nature of Blood**
   1. Blood—highly complex mixture of cells, enzymes, proteins, and inorganic substances
      - Plasma—fluid portion of blood; composed primarily of water
         - 55% of blood content
      - Suspended in plasma; 45% of blood content
         - Erythrocytes (red blood cells or RBC)—transports O and CO$_2$
         - Leukocytes (white blood cells or WBC)—immune system, kill foreign bacteria, cleaning the blood
         - Platelets—used in clotting
      - Serum—pale yellowish liquid that separates from the blood when a clot is formed
   2. Red blood cells transport oxygen from lungs to body tissues and remove carbon dioxide from tissues by transporting it back to lungs
      - On the surface, millions of characteristic chemical structures called antigens (usually a protein, that incites the formation of antibodies)
      - More than 15 blood antigen systems have been identified; A-B-O and Rh systems are the most important
   3. A-B-O system
      - Type A blood—simply indicates that each RBC has A antigens
         - Approximately 40% of general population
         - Produces anti-B antibodies
      - Type B blood—each RBC has B antigen
         - Approximately 12% of gen. pop.
      - Type AB blood—contains both A and B antigens
o Approximately 5% of gen. pop.
   ▪ Type O blood—neither A nor B antigens
   o Approximately 43% of gen. pop.
   o Considered the universal blood type and is the only blood that can be transfused to patients with other blood types
   o However, can only receive Type O blood in transfusions
   ▪ 80% of the population are secretors which means that their blood type antigens are found in bodily fluids other than blood
4. Rh factor—also called D antigen
   ▪ RBCs that have D antigen are said to be Rh positive
   ▪ RBCs that do not have D antigen are Rh negative
   ▪ Out of 100 blood donors, 84 are Rh + and 16 are Rh -
5. II. “Is the substance blood?”—Blood synthesis
   A. SCIENCE—Redox reactions, catalysts
   B. Making synthetic blood activity (?)
   C. Is it blood? activity
III. “Is the substance human blood?”
IV. “If it is human blood, to what blood group does it belong?”
   A. Fundamental principle—for every antigen, there exists a specific antibody
   B. Terminology
      ▪ Agglutination—the clumping together of blood cells
      ▪ Agglutinin—an antibody in plasma that promotes agglutination
      ▪ Agglutinogen—a substance in red blood cells that acts as an antigen and incites the production of agglutinin
      ▪ Antibody—a substance in blood that reacts with a specific antigen, causing blood cells to clump together
      ▪ Antigen—a substance that incites the formation of antibodies; a protein on the surface of red blood cells
      ▪ Hemoglobin—the oxygen carrying coloring matter of red blood cells
   C. Blood typing activity (milk & vinegar)
V. Blood spatter
   A. SCIENCE—projectile motion, gravity, trigonometry
   B. Blood spatter activities
Forensic Serology Module—Lecture Materials

I. Serology Overview
   A. Terminology
      • Agglutination—the clumping together of red blood cells by the action of an antibody
      • Antibody—a substance in blood that reacts with a specific antigen, causing blood cells to clump together
      • Antigen—a substance that incites the formation of antibodies; a protein on the surface of red blood cells
      • Antiserum—blood serum in which there are specific antibodies
   B. History
      1. Before 1900s—physicians had attempted to transfuse blood
         ▪ Resulted in coagulation causing instantaneous death
      2. 1901—Karl Landsteiner—discovered blood typing
         ▪ Recognized that all human blood was not the same; distinguishable by its group or type
         ▪ Won Noble Prize in 1930
      3. 1930s—the Rh factor was recognized
      4. 1990s—the advent of DNA technology dramatically altered the approach forensic scientists take toward the individualization of bloodstains and other biological evidence
   C. Nature of Blood
      1. Blood—highly complex mixture of cells, enzymes, proteins, and inorganic substances
         ▪ Plasma—fluid portion of blood; composed primarily of water (~90%)
           ▪ 55% of blood content
         ▪ Suspended in plasma; 45% of blood content
           ▪ Erythrocytes (red blood cells or RBC)—transports $O_2$ and $CO_2$
           ▪ Leukocytes (white blood cells or WBC)—immune system, kills foreign bacteria, cleaning the blood
           ▪ Platelets—(thrombocytes) used in clotting
         ▪ Serum—pale yellowish liquid that separates from the blood when a clot is formed
      2. Red blood cells transport oxygen from lungs to body tissues and remove carbon dioxide from tissues by transporting it back to lungs
         ▪ On the surface, millions of characteristic chemical structures called antigens (usually a protein, that incites the formation of antibodies)
         ▪ Antibody—substance that reacts with a specific antigen, causing blood cells to clump together
         ▪ More than 15 blood antigen systems have been identified; A–B–O and Rh systems are the most important
   D. Paternity Suit activity (criminalistics case report form)—see lab activity
      • Students use Punnett squares to determine the likelihood of an illegitimate child in hypothetical paternity cases
II. "Is the substance blood?"—Methods forensic scientists use to detect blood

A. Luminol

1. What is luminol?
   - Yellowish-white crystalline compound, \( C_8H_7N_3O_2 \)
   - Produces a brilliant bluish luminescence when treated in an alkaline solution
     with an oxidizing agent
     - Similar process occurs in fireflies and "glow" sticks

2. How is luminol used by forensic scientists?
   - Tiny particles of blood will cling to most surfaces for years even after
     being cleaned (supposing that "heavy-duty" cleaners were not utilized)
   - Investigators use luminol to detect the presence of blood at a crime scene
   - Luminol produces characteristic blue glow when it is catalyzed by the iron
     found in hemoglobin (oxygen-carrying protein) in blood
   - However, luminol can destroy other evidence at crime scenes
     - Used only after exploring other options
     - It is not as prevalently utilized as portrayed on TV
   - In addition, luminol can be catalyzed by other materials (such as metals—
     Fe, Cu, Cr, etc.)
     - Therefore, subsequent testing may be necessary

3. A deeper scientific understanding to the reaction process—solutions, catalysts, redox reactions, excitation of electrons, and conservation of energy
   - Solutions: To prepare luminol for blood detection use, one must make a
     homogeneous solution that includes luminol powder, hydrogen peroxide
     (\( \text{H}_2\text{O}_2 \)), a hydroxide source (\( \text{OH}^- \)), and other chemicals.
   - Catalysts: If hemoglobin and the luminol mixture come into contact, the
     iron in the hemoglobin acts as a catalyst and accelerates the reaction
     between the hydrogen peroxide and the luminol.
     - Reaction occurs slowly (to the unaided eye, difficult to detect)
       without the presence of a catalyst (e.g. Fe, Cu, Cr, Ni, etc.)
   - Redox: In the oxidation reaction, the luminol gains oxygen atoms to form
     the compound 3-aminophthalate.

\[
\begin{align*}
2 \text{OH}^- + \text{luminol} & \quad \xrightarrow{\text{H}_2\text{O}_2, \text{microperoxidase; } \text{pH}=10} \quad \left[ \begin{array}{c}
\text{O} \\
\text{NH}_2 \\
\text{NH} \\
\end{array} \right] + \text{N}_2 + 2\text{H}_2\text{O} \\
\end{align*}
\]

- Electrons: The reaction causes the electrons in the oxygen atoms to be
  boosted to higher orbitals.
  - The electrons quickly fall back to the lower energy level, emitting
    the extra energy as a light photon (we see a "glow")
- Energy: In this reaction, the reactants have more energy than the products
  - Total energy of the system must be conserved
  - Extra energy in the reactants is converted to light energy

B. Fluorescein—similar chemiluminescent process to luminol; procedure slightly different

C. Phenolphthalein—color test which turns fuchsia pink; potatoes and horseradish can provide a false positive

D. Benzidine color test—because of hazards no longer used

E. Kastle-Meyer color test—uses phenolphthalein and other chemicals to determine the presence of blood; turns fuchsia pink

F. Is it Blood Activity—see lab activity for detailed descriptions
  - Students will use luminol and a variety of red substances to predict the presence of “blood”
  - Uses synthetic blood; recipe in teacher materials

III. “Is the substance human blood?”—uses human antiserum

A. Precipitin test
  - Positive test would detect a white line formed at the interface of the blood sample and human antiserum
  - Very sensitive test requiring only a small sample size
  - Can be used on stains dried for 10-15 years

B. Gel Diffusion
  - Antibodies and antigens will attract each other
  - If unknown is human, sample and antiserum (on a starch gel plate) will migrate and form a line of agglutination

C. Electrophoresis
  - Electrical charge on the plate causes samples to migrate
  - Reaction between sample and antiserum will result in a line of precipitation

IV. “If it is human blood, to what blood group does it belong?”

A. A-B-O System
  1. Type A blood—simply indicates that each RBC has A antigens
     - Approximately 40% of general population
     - Produces anti-B antibodies
  2. Type B blood—each RBC has B antigen
     - Approximately 12% of general population
     - Produces anti-A antibodies
  3. Type AB blood—contains both A and B antigens
     - Approximately 5% of general population
     - Produces neither anti-A nor anti-B antibodies
  4. Type O blood—neither A nor B antigens
     - Approximately 43% of gen. pop.
     - Produces both anti-A and anti-B antibodies
     - Considered the universal blood type and is the only blood that can be transfused to patients with other blood types
• However, can only receive Type O blood in transfusions
5. 80% of the population are secretors, meaning their blood type antigens are found in bodily fluids other than blood

B. Rh Factor—also called D antigen
• RBCs that have D antigen are said to be Rh positive
• RBCs that do not have D antigen are Rh negative
• Out of 100 blood donors, 84 are Rh + and 16 are Rh -

C. Blood Typing Tests
• Fundamental principle—for every antigen, there exists a specific antibody
• Type A will agglutinate with anti-A serum
• Type B will agglutinate with anti-B serum
• Type AB will agglutinate with both anti-A and anti-B serum
• Type O will not agglutinate with either anti-A or anti-B serum

D. A-B-O Blood Typing Simulation Activity—see lab activities for detailed description
• Students will determine the blood type of unknown samples
• Uses milk, vinegar, water, and synthetic blood to simulate blood typing

V. Blood spatter
A. What bloodstains can reveal
1. Blood drop behavior in the air depends on how it left the body (e.g. gunshot, blunt force, or sharp instrument)
2. Blood spatter patterns (BSP) depends on:
   • Amount of blood
     o Mist (tiny droplets): < 1 mm diameter = tremendous forces (e.g. gunshot at close range)
     o Fine droplets: < 3 mm diameter = forces greater than gravity (e.g. gunshot)
     o Medium droplets: 3-6 mm diameter = due to gravity and other forces (e.g. blunt trauma)
     o Large droplets: > 6 mm diameter = due to gravity alone
   • Angle of impact
     o Greater the angle, the more elliptical the shape
     o Determines direction
   • Distance blood dropped
     o Greater distance the larger the spatter diameter
     o Only useful for the first 5 to 6 feet (1.5-1.8 m) from impact
3. Degree of spatter from a single blood drop depends primarily on the type of surface on which it falls
   • Coarser the surface, the more likely it will rupture and spatter
4. Influence of velocity on spatter:
   • Velocity of freely falling drop of blood is influence by the force of gravity
     o Terminal (low) velocity for a drop of blood = 7.6 m/s (or 25 ft/s)
   • Medium velocity impact blood spatter—produced by external, medium velocity forces
According to Newton’s laws of motion (F=ma), if more force is involved there will be an acceleration of the blood drop (i.e. gunshot)
  - Low velocity changes to medium velocity (> 7.6 m/s)
  - Medium velocity changes to high velocity (> 30 m/s or 100 ft/s)

5. Conclusions about velocity and impact should not be drawn from very small bloodstains
  - Could represent castoffs (satellites of larger drops)

6. Spatters can indicate the position of victim and perpetrator
  - Small, independent spatters usually have a uniform taper, in the shape of a teardrop.
    - Tail of the teardrop always points away from the direction of impact
  - Small castoffs resemble tadpoles and tend to be longer and narrower than the teardrop
    - Sharper end always points back toward the direction of impact

B. Blood spatter Activity—see lab activity for detailed description
  - Students use synthetic blood to investigate blood spatter patterns.
  - Students will utilize graphing techniques to determine relationships between height, angle of impact, and drop dimension ratios.
Further information and resources:

- **Forensic Science.** Stuart H. James and Jon J. Nordby. CRC Press. 2005
- **Criminalistics.** Richard Saferstein. Pearson/Prentice Hall. 2004
- **Practical Crime Scene Processing and Investigation.** Ross M. Gardner. CRC Press. 2005
- www.susq-town.org/evans/forensicbgs/bloodsplatterevidence05notes.pdf
  Ms. Evans, Susquehanna Township High School 2005
- http://people.howstuffworks.com/luminol1.htm
  How Stuff Works, Inc. 1998-2005
  The American National Red Cross, 2003
  "Development of a Synthetic Blood Substitute for use in Forensic Science Teaching." Joanne Millington, MSc. Liverpool University for The Higher Education Academy Physical Science Center.
- http://library.thinkquest.org/04oct/00206/bloodanal.htm
  Thinkquest Entrant October 2004
- http://www.nvps.net/npsnhs/Curriculum%20Maps/blood%20splatter%20lab.doc
  Brain Bollone. Northview High School. Grand Rapids, MI
\[ 2 \text{OH}^- + \text{luminol} \xrightarrow{\text{H}_2\text{O}_2, \text{microperoxidase}, \text{pH}=10} \text{3-amino phthalate} + \text{N}_2 + 2\text{H}_2\text{O} \]
Synthetic Blood

Materials for 100 mL:
- Flour (superfine, self-rising) 3.25 g
- Sodium chloride 0.25 g
- Glycerol (glycerin) 0.25 mL
- Corn syrup (light, Karo ®) 0.25 mL
- Deionized water 100.0 mL
- Food coloring (red and blue)

Equipment:
- 250 mL beaker
- Hot plate
- Graduated cylinders (10 mL and 100 mL)
- Stirring rod or magnetic stirrer and plate
- Thermometer (Celsius)
- Pipette

Procedure:
1) Measure all powders and liquids.
2) Pour 48.0 mL of deionized H₂O into a 250 mL beaker. Place on a hot plate and begin to heat on low. Use the magnetic stirrer to stir or using a stirring rod, stir often.
3) Add NaCl to the beaker.
4) Pipette 5 drops (0.25 mL) of the glycerol into the beaker.
5) Pipette 5 drops (0.25 mL) of the corn syrup into the beaker.
6) Slowly add the flour to the beaker.
   *Do not add all at once. This will cause clumping.
7) Add 5 drops (0.25 mL) of red food dye to the mixture.
   *Make sure all of the flour is homogeneously dispersed before proceeding.
8) Frequently stirring, heat the "blood" mixture to boiling (approximately 90.0°C). Allow to boil for 1-2 minutes (mixture will be very thick). Remove from hot plate and allow to cool.
9) While the "blood" is cooling, make a solution of diluted blue dye. Using a graduated cylinder, add 1 drop of blue dye to 10.0 mL of H₂O. Use a pipette to dispense approximately 45 drops (2.25 mL) to the "blood." Stir completely.
10) While stirring, add 20 drops (1.00 mL) of concentrated red dye.
11) Add 20.0 mL of H₂O to the "blood." Stir until a homogenous consistency is achieved. Store in a refrigerator for stability.

*TIP: Amount of H₂O and dye may vary depending on subjective opinion for viscosity and color.
Suggestions & Options:

- **Suggestions:** you might store the "blood" in a refrigerator for stability (the flour tends to settle out over time).

- **Is It Blood? Activity:** To react with luminol, add 10 mL of 0.08 M CuSO₄·5H₂O to the synthetic blood mixture. Stir well. The copper will now catalyze the luminol to produce a brilliant chemiluminescence under low lighting.
  - To prepare 0.08 M CuSO₄·5H₂O, add 0.2 g of copper (II) sulfate pentahydrate to 10 mL of deionized water (molar mass of CuSO₄·5H₂O = 249.6 g/mol)

- **A-B-O Blood Typing Activity:** To prepare unknowns (adjust color with red food dye)
  - #1—synthetic blood to simulate Type O blood
  - #2—synthetic blood and milk mixture (1:1) to simulate Type A blood
  - #3—synthetic blood and vinegar mixture (2:1) to simulate Type B blood
  - #4—two containers—one synthetic blood/milk & one synthetic blood/vinegar to simulate Type AB blood

- **Blood Spatter Activity:** See synthetic blood procedure
Is it Blood?
Laboratory Activity

Purpose/Objective  The student will...
• Identify substances as "blood" based on laboratory observations.
• Utilize laboratory techniques and demonstrate proper safety practices.
• Work cooperatively in a group setting.

Hypothesis
How can blood accurately be detected? Can blood be assumed by the presence of a red liquid or stain?
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Materials
• 11 unknown samples
• Pipette
• Spray bottle with luminol solution

Safety  Luminol is an irritant. Use in a well ventilated area. Wear goggles for eye protection. Wear gloves for skin protection.

Procedure
PART ONE:
1) Make qualitative observations about each sample. Record in the data table.
2) Use a pipette to drop 2 drops of each sample onto a 10 cm x 10 cm portion of a paper towel. Label each paper towel with the letter of the unknown sample.
3) Separately place each paper towel into the fume hood or box and spray with luminol. Do not saturate paper towel.
4) Record your observations in the data table. Speculate on the possibility of the substance being blood.
5) Dispose of the paper towels into the proper receptacle.

PART TWO:
6) Your teacher has some unknown samples on paper towels that have been washed and rinsed. Record your observations.
7) Test to see if any of them had been blood. Record your observations.
**Observations**

**PART ONE**

<table>
<thead>
<tr>
<th>Unknown Sample</th>
<th>Observations (describe visual appearance and reaction with luminol)</th>
<th>Blood?</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
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**PART TWO**

<table>
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<th>Unknown Sample</th>
<th>Observations (describe visual appearance and reaction with luminol)</th>
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<tr>
<td>X</td>
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<td>Z</td>
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</table>
Analysis & Conclusion

1) Explain how you determine which sample(s) was blood.
_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________

2) Are all red liquids or stains blood? Explain.
_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________

3) If a substance catalyzes the reaction of luminol, is it definitively blood? Explain.
_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________

4) If a perpetrator visually cleans up blood after a crime, can the blood be detected? Explain.
_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________

Extension & Application

1) Propose an experiment to perform on the samples that you determined were blood which would further reinforce this hypothesis. After instructor approval, perform your investigation and report on your results.

2) Research other methods used to detect the presence of blood. Identify which, in any, of these other tests can “prove” the existence of blood.
Teacher Suggestions & Expected Results

Prep. time: 20-30 minutes
Lab time: 45-60 minutes

- To prepare suggested unknown samples:
  - A—catsup
  - B—spaghetti sauce
  - C—tomato juice
  - D—Tobassco sauce
  - E—red laytex paint
  - F—beet juice
  - G—synthetic blood (recipe included)
  - H—water colored with red food dye

  Place a small amount of each sample into a labeled clear vial or container.

- To prepare the unknown samples:
  - X—repeat of A through F or H
  - Y—synthetic blood
  - Z—copper (II) sulfate solution (few drops of 0.2 g CuSO₄/10 mL H₂O)

  Stain a 10 cm x 10 cm portion of a paper towel with each sample. After 3-5 minutes, rinse the paper towels with water and allow them to dry.

- Suggested preparation for the luminol
  - 0.2 g luminol powder
  - 10.0 g Na₂CO₃
  - 180 mL deionized H₂O
  - 180 mL 3% H₂O₂

  Can be bought as a solution or prepare as directed. For storing luminol, place in a brown container. Each lab group should have a small spray bottle of luminol (contact local pharmacy for fine misting spray bottle)

- If using fume hood, the room must be darkened to view luminescence. To prepare the modified box, use a shoe box or another cardboard box with a lid. Cut an opening a one end. Students will insert the sample, spray with luminol, quickly fasten the lid, and look through the opening to view the luminescence.
A-B-O Blood Typing
Laboratory Simulation Activity

Purpose/Objectives: The student will...
- Identify various blood types.
- Utilize laboratory techniques to determine and analyze blood types.
- Recognize the percentages of blood types that occur in the general population.

Hypothesis:
Which blood type is the most common blood type? Which blood type is the least common? Why?

Materials:
- Test tubes and rack
- Pipette or graduated cylinder, 10 mL
- Stirring rod
- Deionized H₂O
- Anti-A and Anti-B serum (in pipettes or dropper bottles)
- Two unknown samples

Safety: Wear goggles for eye protection. Proceed with caution when using glassware.

Procedure:
1) Using a glass marking utensil, label two test tubes “anti-A” and “anti-B”.
2) Measure approximately 1 mL of your unknown sample into both test tubes.
3) Using the anti-serums, pipette 20 drops (~ 1 mL) of each serum into the appropriately labeled test tube (i.e. anti-A serum into the anti-A test tube).
4) Stir using the stirring rod (be sure to clean it before using it in a different test tube).
5) Check each test tube for agglutination and record your observations. A positive reaction would agglutinate and a negative reaction would not agglutinate.
6) Determine the blood type of your unknown sample.

<table>
<thead>
<tr>
<th>Unknown #</th>
<th>Reaction (+ or -) &amp; Observations</th>
<th>Unknown #</th>
<th>Reaction (+ or -) &amp; Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td></td>
<td>Anti-A</td>
<td></td>
</tr>
<tr>
<td>Anti-B</td>
<td></td>
<td>Anti-B</td>
<td></td>
</tr>
</tbody>
</table>

Analysis & Conclusion:
1) Blood type of Unknown # _______ is ______________.
   Blood type of Unknown # _______ is ______________.
2) Indicate your results with the reported class data (on board). Complete the following table and calculate the percentages.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>AB</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentages</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Your teacher will provide you with the percentages of blood types in the general population.
- How does the class results compare with the percentages of blood types in the general population?

__________________________________________________________________________

__________________________________________________________________________

3) Make a bar graph representing the occurrence of the blood types in your class data.
Extension & Application:

1) Research the evolutionary basis for blood types. Prepare a report and include graphs.
2) Research the role of genetics in determining blood type. Prepare a report.
3) Research the blood type occurrences in secluded, segregated populations. Prepare a report and include graphs.

Teacher Suggestions and Expected Results

Prep. time: excluding preparation of synthetic blood, 20-30 minutes
Lab time: 20-30 minutes

This activity will allow students to explore the events of laboratory testing of blood types without the hazards that come with using human blood. The samples are milk, vinegar, and deionized water and they will be used to simulate acceptance/rejection in blood. Milk and vinegar react, causing clumping (agglutination) of the "antigen" and "antibodies." Students will work cooperatively to determine the blood type of their unknown sample and work collectively to analyze the class results.

- Prepare unknowns (adjust color with red food dye)
  o #1—synthetic blood to simulate Type O blood
  o #2—synthetic blood and milk mixture (1:1) to simulate Type A blood
  o #3—synthetic blood and vinegar mixture (2:1) to simulate Type B blood
  o #4—two containers—one synthetic blood/milk & one synthetic blood/vinegar to simulate Type AB blood
    ▪ The two containers will "act" as one and both will be treated with each anti-serum.
    ▪ You will most likely have to explain this simulated AB to those lab groups that receive unknown #4.

- Prepare anti-serums (colored with yellow food coloring)
  o Anti-A—vinegar
  o Anti-B—milk

- Synthetic blood recipe included.

- Suggestion: make the unknowns reflect the percentage of the population having these blood types. For a class of 7 or 8 groups, 7 Type O, 5 Type A, 2 Type B, and 1 Type AB

- After the students have determined the identity of their unknown sample(s), have them record their information on the board (similar to the table in analysis) to assist in gathering group data. When all data is reported, the students will calculate the percentages of each blood type (this should reflect the given percentages, and samples will be prepared with that in mind) and create a bar graph.

- You will need to provide the percentages for the general population (at the appropriate time)
  o Type O ≈ 43%
  o Type A ≈ 40%
  o Type B ≈ 12%
  o Type AB ≈ 5%
FIGURE 12-1  Red blood cells containing B antigens are agglutinated or clumped together in the presence of B antibodies.

Red blood cells containing A antigens will not combine with B antibodies.

Red blood cells containing B antigens are agglutinated or clumped together in the presence of B antibodies.
Criminalistics Case Report Form

Brief Background: In 1901, Karl Landsteiner made one of the most significant discoveries of the century—the typing of blood. It was Landsteiner who first recognized that all human blood was not the same; instead he found blood to be distinguishable by its group or type. Out of Landsteiner’s work came the classification system that we presently call the A-B-O system. By 1937, the Rh factor in blood was demonstrated.

The problem of identifying most blood factors in itself is a difficult task. As blood dries, some of its characteristic blood factors are destroyed; and as the stain continues to age, the destruction slowly extends to the basic A-B-O system. It is up to the serologist—a person who tests specific antigen and serum antibodies—to pursue the detection and identification of various blood characteristics.

In humans, the population distribution of blood types varies with location and race throughout the world. In the United States, a typical distribution is as follows:

<table>
<thead>
<tr>
<th></th>
<th>O</th>
<th>A</th>
<th>B</th>
<th>AB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>43%</td>
<td>42%</td>
<td>12%</td>
<td>3%</td>
</tr>
</tbody>
</table>

In order to be a specific blood type, you must receive half of your genetic characteristics from your mother and the other half from your father. Because of this basic understanding of human genetics, it is possible to determine (with some accuracy) if a child was conceived by the “natural” parents. Each blood type has the following genetic combination(s).

<table>
<thead>
<tr>
<th></th>
<th>O</th>
<th>A</th>
<th>B</th>
<th>AB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OO</td>
<td>AA or AO</td>
<td>BB or BO</td>
<td>AB</td>
</tr>
</tbody>
</table>

Purpose: You are a legal assistant who has been given the following paternity cases to review. It is your job to screen each case to see if there is any validity to the claim of an “illegitimate” child and then make your recommendations to the lawyers for whom you work for.

Procedure: Proceed with your recommendations, indicating a “yes” or “no” for each potential case. Also, write out the genetics make-up (letter combinations) for each case indicating why you choose “yes” or “no”.
Case 1:
Mother: A
Father: B
Grandmother: O
Grandfather: AB
Children: None
Child in Question: O

Case 2:
Mother: AB
Father: A
Grandmother: A
Grandfather: A
Children: A six-year-old girl with "AB" and a two-year-old boy with "A"
Child in Question: O

Case 3:
Mother: B
Father: B
Grandmother: B
Grandfather: AB
Children: A three-year-old boy and a two-year-old boy both with "B"
Child in Question: O

Group 4:
Mother: O
Father: O
Grandmother: A
Grandfather: B
Children: None
Child in Question: O

Group 5:
Mother: B
Father: A
Grandmother: B
Grandfather: A
Children: A six-year-old girl with "B"
Child in Question: A

Group 6:
Mother: O
Father: O
Grandmother: B
Grandfather: B
Children: None
Child in Question: A

Group 7:
Mother: AB
Father: AB
Grandmother: A
Grandfather: AB
Children: A thirteen-year-old girl with "AB", a nine-year-old girl with "AB", a seven-year-old boy with "B", and a three year old with "AB"
Child in Question: A
Expected results:

Case 1: Not valid. It is possible for the mother to have an “AO” blood type and the father to have a “BO” blood type. This gives them a $\frac{1}{4}$ possibility to have a child with an “O” blood type.

Case 2: Valid. The mother has an “AB” blood type. Regardless of the father’s blood type, it is impossible for the child to receive an “O” from both parents.

Case 3: Valid. The father’s grandparents both have an “AB” blood type. Since the father has a “B” blood type, it is only possible that it is comprised of two “B’s” from his parents. Thus, it is impossible for the father to pass an “O” to his child.

Case 4: Not valid. Both parents inherited an “O” blood type. Since only “O’s” are passed on, the child will have a 100% chance to have an “O” blood type.

Case 5: Not valid. The mother has the potential to have a “B” blood type consisting of a “BO” makeup. The father has the potential to have an “A” blood type consisting of an “AO” or an “AA” makeup. Combined, there is a high probability the child will have an “A” blood type.

Case 6: Valid. Both parents inherited an “O” blood type. It is only possible for them to pass “O’s” to their children. Thus it is impossible to pass on an “A” blood type.

Case 7: Not valid. Both parents inherited an “AB” blood type. There is a $\frac{1}{4}$ chance that the child may inherit two “A’s”. This would give the child an “A” blood type.
Blood Spatter
Laboratory Activity

Purpose/Objectives: The student will...
• Observe the effect of height on spatter patterns.
• Explore the relationship between angle of impact and pattern formed.
• Utilize graphing techniques to analyze data.

Hypothesis: Predict how height will affect spatter patterns. Predict how angle of impact will affect spatter patterns.

Safety: Synthetic blood may stain clothing and skin. It is suggested to wear lab aprons to protect clothing.

Materials:
• Synthetic blood
• Paper, unlined
• Tape
• Glass pipette
• Metric ruler and meter stick
• Base (book, board, cardboard, etc.; to vary angles)
• Protractor
• Graph paper

Procedure:
1) Tape a piece of paper on a flat, hard surface. See Chart and Notice Heights and Angles.
2) Use the pipette to drop one droplet of blood onto the paper. Record any observations. Label the spatter with the corresponding height and angle.
3) Position the paper to accommodate ensuing drops for the next height or angle.
4) Continue the process, using different pieces of paper as necessary, for the indicated angles (0°, 20°, 40°, & 60° relative to the horizontal) and indicated heights.
5) Carefully, measure the length (l) and width (w) of each blood spatter and record in the data table.
   **Be careful as you measure the spatters; synthetic blood takes a while to dry! Measure only the elliptical impact pattern (i.e. do not include drip into the length measurement!)**
6) Divide the width (w) by the length (l) and record this number to the nearest 1/1000th in the appropriate area (values should be greater than 1.000).
7) Create two graphs. On the first graph, the x-axis represents the angle of impact and the y-axis represents the ratio of width to length (w/l). Using different symbols or colors, plot the data for each height.
8) On the second graph, the x-axis represents the height and the y-axis represents the ratio of width to length (w/l). Using different symbols or colors, plot the data for each angle.
9) Appropriately title each graph.
<table>
<thead>
<tr>
<th></th>
<th>0°</th>
<th>20°</th>
<th>40°</th>
<th>60°</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>15 cm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>w=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>w/l=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Observations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>45 cm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>w=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>w/l=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Observations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>75 cm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>w=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>w/l=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Observations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>105 cm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>w=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>w/l=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Observations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>135 cm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>w=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>w/l=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Observations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>165 cm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>w=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>w/l=</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Observations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Analysis & Conclusion:

1) Describe your resulting graphs. If there is a relationship, identify the relationship (e.g. linear, inverse, squared, etc.).

_____________________________________________________________________
_____________________________________________________________________

2) Explain the effect of angle on the width/length ratio.

_____________________________________________________________________
_____________________________________________________________________

3) Explain the effect of height on the width/length ratio.

_____________________________________________________________________
_____________________________________________________________________

4) Explain why there is a difference between spatter patterns on a flat surface compared to angular surfaces.

_____________________________________________________________________
_____________________________________________________________________

   o Is there more or less difference as the angle increases? Why?

_____________________________________________________________________
_____________________________________________________________________

5) Utilize your graphs to answer the following:
   o For a height of 75 cm, what would be the ratio at 30°?_____________________
   o For a height of 105 cm, what would be the ratio at 45°?_____________________
   o For a height of 15 cm, what angle would produce a ratio of 0.900?_____________
   o For a height of 135 cm, what angle would produce a ratio of 0.700?___________

6) How would a crime scene investigator utilize this information about blood spatter patterns to reconstruct a crime scene?

_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________
**Teacher Suggestions & Expected Results:**

Prep time: excluding the preparation of the synthetic blood, 15-20 minutes
Lab time: 60-70 minutes

- Explain to the students that this is a simplified version of the information forensic scientists understand about blood spatter. The topic of blood spatter analysis is actually more complex and takes into account secondary spatter, cast off spatter, time, surfaces, and a variety of other variables. This gives an idea of how this information can be collected and interpreted.
- To reduce time depended and other systematic errors, instruct the students to take measurements randomly. Meaning: don’t do 15 cm @ 20° then 45 cm @ 20° then 75 cm @ 20° and so on. Instead do 15 cm @ 20° then 135 @ 40° then 75 @ 0° and so on. Doing this is better experimental science. However, this will make it take longer!
- Instruct the students to make their graphs as large as possible, using two pieces of graph paper instead of crowding two graphs onto one paper. The scale on the axis should maximize the data (i.e. not necessarily will 0, 0 be on the graph).
- Measuring the resultant stain dimensions: width is the widest portion of the spatter and length is the longest portion of the elliptical nucleus of the spatter.
- Synthetic blood recipe included.
- Resource/money saving tip: use left over paper from “copier mistakes” (usually found in teacher workrooms) to save paper.
- Student suggested suggestion: use paper towels instead of paper; it dries faster (however, the measurements become more subjective because of bleeding)
- Cover the student work area with newspaper to avoid messes on the floor.
- To create the angles, tape two sheets of cardboard together and place a book in the middle. Adjust the book to create the desired angles. Clip or tape the paper to the face of the cardboard, making sure there are no creases in the paper.
- A trigonometric relationship exists between the angle of impact and stain dimensions such that: $\sin \theta = \text{width}/\text{length}$ (where $\theta$ is the angle of impact or inclination of the surface)
- Angle decreases the ratio while height does not change the ratio.
<table>
<thead>
<tr>
<th>height</th>
<th>0</th>
<th>15</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>1.0</td>
<td>1.071</td>
<td>0.878</td>
<td>0.817</td>
</tr>
<tr>
<td>45</td>
<td>0.992</td>
<td>1.083</td>
<td>0.967</td>
<td>0.726</td>
</tr>
<tr>
<td>75</td>
<td>0.984</td>
<td>1.059</td>
<td>0.938</td>
<td>0.729</td>
</tr>
<tr>
<td>105</td>
<td>1.017</td>
<td>1.091</td>
<td>0.783</td>
<td>0.659</td>
</tr>
<tr>
<td>135</td>
<td>1.017</td>
<td>1.061</td>
<td>0.8</td>
<td>0.746</td>
</tr>
<tr>
<td>165</td>
<td>1.077</td>
<td>1.042</td>
<td>0.714</td>
<td>0.795</td>
</tr>
</tbody>
</table>
Blood dropped vertically from a height of 50 inches (1.3 meters) onto a flat surface shows a radiating, or bursting, effect. The stain is 1 inch (2.5 centimeters) in diameter.

The same amount of blood as in a, but dropped vertically onto a flat surface from a height of 8 inches (20 centimeters), makes a smaller stain (3/4 inch [about 2 centimeters]) with a wavy edge.

Oval stain has spattering showing two or three spikes (splashes). Broad portions of the splashes indicate the point at which the blood first struck obliquely on a flat surface and thus show the direction from which it came.

Stain shows two oval spots like an exclamation mark and indicates a left-to-right direction of blood drop.