CSI: St.Lawrence
Laboratory Activity
Properties and Forensic Uses of Blood
February 21, 2008

Objectives:
In this laboratory exercise, you will explore some of the properties and forensic uses of blood.

Background:
Refer to the sections in Chapter 14 of your textbook that deal with blood (pages 213-222).

Note:
All objects that come into contact with human blood must be disposed in bleach water.

Assignment – Due February 28, 2008

One typed report per team
1. a. Compare red blood cells and white blood cells. Consider what you would use to differentiate between the two types of cells. (ex: number or proportion of cells, shapes, color)
   b. Describe the differences you observed between normal blood and sickle-cell anemic blood.
   c. Describe the differences you observed between normal blood and leukemia blood.
2. a. List the blood type of all members of the team.
   b. Identify the possible gene combinations for each individual’s blood type.
3. a. Provide a table of your results
   b. Based on your results and observations, determine if the samples tested are blood. Justify your decision using the data gathered during the lab activity.
4. Compare the three presumptive tests for blood. Describe one disadvantage and one advantage of each test.
Microscopic observation of blood cells

Materials:
- microscope
- identification key to the parts of the microscope
- prepared slides of human blood cells
  - normal
  - sickle cell anemia
  - leukemia

Procedure:
1. Position yourself at one of the microscopes set up in the classroom. Each microscope will have a prepared slide of human blood cells in viewing position with at magnification of 400X (i.e. 400-times larger than life-size).
2. Adjust the distance between the eyepieces until you are able to see a single, circular microscopic image when looking into the microscope. You may also need to adjust the distance between your eyes and the microscope.
3. Examine the cells in your field of view. Use the fine adjustment knob to adjust the focus if necessary. If desired, you may use the mechanical stage control knobs to examine other areas of the slide.
4. If you are unable to clearly see blood cells, please ask for assistance.
5. Examine three types of blood cell preparations
   a. normal cells: identify red blood cells and white blood cells. What observable characteristics would you use to differentiate between the two types of cells?
   b. sickle cell anemia cells: identify the differences between this blood sample and a normal blood sample.
   c. blood cells from and individual with leukemia: identify the differences between this blood sample and a normal blood sample.

Assignment – Microscopic observation of blood cells
Compare red blood cells and white blood cells. Consider what you would use to differentiate between the two types of cells. (ex: number or proportion of cells, shapes, color)
Describe the differences you observed between normal blood and sickle-cell anemic blood.
Describe the differences you observed between normal blood and leukemia blood.
Determine your blood type

**Note:** do NOT attempt to perform this procedure if the sight of blood makes you feel dizzy, nauseous or faint.

**Materials:**
- clean microscope slide
- wax pencil
- blood-typing antibodies
- light box
- alcohol wipes
- blood lances and automatic lancer
- bleach solution for slide and lance disposal
- toothpicks

**Procedure:**
1. Use a wax pencil to mark a clean microscope slide with 3 circles. Label the circles A, B, and Rh.

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A   B   Rh
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2. Use an alcohol wipe (or cotton swab and rubbing alcohol) to disinfect the finger you wish to use to provide a blood sample. Discard the wipe and its wrapper.

3. When your finger is no longer wet with alcohol, your instructor will use the automatic lancer to prick your finger.

4. Squeeze one drop of blood into each of the circles marked on your microscope slide.

5. Without touching the blood drop, your instructor will add one drop of anti-A solution to the blood in circle A, one drop of anti-B solution to the blood in circle B, and one drop of anti-Rh solution to the blood in circle Rh.

6. Use a toothpick to mix your blood with the anti-A solution. Immediately discard this toothpick in the bleach solution.

7. Mix the two remaining blood-antibody mixtures, using separate toothpicks for each solution and discarding the toothpicks immediately after use.

8. Place your slide on the light box and observe after 1-2 minutes.
   a. If clumping occurs in circle Rh, your blood is Rh positive
   b. If no clumping is observable in circle Rh, your blood is Rh negative
   c. If clumping occurs in circle A, your blood is type A
   d. If clumping occurs in circle B, your blood is type B
   e. If clumping occurs in both circles A and B, your blood is type AB
   f. If no clumping is observable in either circle A or B, your blood is type O

9. After you have determined your blood type, discard your slide into the bleach solution.

**Assignment – Determine your blood type**

List the blood type of each member of your team.
Identify the possible gene combinations for **each** team member.
Presumptive tests for blood

Samples of stains present at a crime scene in the biology classroom have been collected. You must determine which of the stains contains blood.

**Kastle-Meyer Test**

**Materials:**
- 3 small filter papers
- distilled water
- unknown sample A (dry)
- Kastle-Meyer reagent
- transfer pipettes
- unknown sample B (dry)
- 3% hydrogen peroxide
- blood sample
- unknown sample C (dry)

**Procedure:**

1. First determine that the filter paper will not react with the Kastle-Meyer solution. This will be your “negative control,” and will be used to determine what the reaction is expected to look like on a non-blood sample. Label the edge of the filter paper with (-).

2. Apply one drop of distilled water to the center of the filter paper.

3. Apply one drop of Kastle-Meyer reagent to the same area of the filter paper.

4. Record any color change.

5. Apply one drop of 3% hydrogen peroxide to the same area of the filter paper.

6. **Wait 2 minutes**, then record any color change.

7. To determine what the reaction is expected to look like when blood is present, prepare a “positive control” by placing a drop of blood on the center of a second piece of filter paper. Label the edge of this filter paper with (+). Repeat steps 3-6.

8. Visually examine the three unknown samples. Record your observations.

9. Fold a circle of filter paper in half to form a half-circle, then fold it a second time to make a quarter-circle. Label the edge of this filter paper “A”

10. Use the point of the folded filter paper to scrape up a small amount of unknown sample A. Unfold the filter paper then repeat steps 3-6.

11. Repeat steps 9 and 10 for unknown samples B and C. Label the filter papers appropriately.

12. When you have completed your analyses, discard the filter papers in the hazardous waste bucket. Do NOT throw the samples into the trash can! Clean your work area before you leave the room.
**Hemastix**

**Materials:**
- Hemastix
- distilled water (negative control)
- blood sample (positive control)
- unknown sample A (liquid)
- unknown sample B (liquid)
- unknown sample C (liquid)
- 5 transfer pipettes
- 5 test tubes

**Procedure:**
1. Briefly dip the colored end of a Hemastix into a test tube of distilled water (negative control).
2. Examine the Hemastix and record its color. Discard the Hemastix after use.
3. Add about 2 cm of distilled water to a test tube.
4. Add 1 drop of the blood sample (positive control) to the distilled water in the test tube. Mix gently.
5. Briefly dip the colored end of a Hemastix into the water-blood mixture. Record the color of the Hemastix. Discard after use.
6. Repeat steps 3 – 5 for the liquid samples A, B and C. To prevent cross-contamination, it is important that you use a different pipette for each sample.
7. When you have completed your analyses, discard the contents of the test tubes into the sink. Rinse the tubes and place in the wash bin. Clean your work area before you leave the room.

**Luminol**

**Materials:**
- Luminol in spray bottle
- distilled water (negative control)
- blood sample (positive control)
- unknown sample A (liquid)
- unknown sample B (liquid)
- unknown sample C (liquid)
- glass plate
- unknown sample D (liquid)
- transfer pipettes

**Procedure:**
1. Place one drop of distilled water (negative control) on a glass plate and use the transfer pipette to spread the drop.
2. Use a piece of paper towel to gently blot the wet sample.
3. The luminol reaction can only be observed in a darkened area; bring the glass plate with the prepared sample to the designated area of the lab.
4. In a darkened room, spray the glass plate with a solution of luminol. Immediately note any color change.
5. Clean the glass plate then repeat steps 1-4 for the positive control and for samples A, B, C and D. To prevent cross-contamination, it is important that you use a different pipette for each sample.
6. When you have completed your analyses, carefully place the glass plate in the wash bin. Clean your work area before you leave the room.

**Assignment – Presumptive tests for blood**

Hand in a table of your results.

Based on your results, which (if any) of the unknown samples is(are) blood? Justify your response using the data you gathered.

Compare the three presumptive tests for blood (Kastle-Meyer, Hemastix, and luminol). Describe one disadvantage and one advantage of each test.
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<th></th>
<th>negative control (water)</th>
<th>positive control (blood)</th>
<th>sample A</th>
<th>sample B</th>
<th>sample C</th>
<th>sample D</th>
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