

Carolina BioKits™

Wright Readi-Stain®

TEACHER'S MANUAL
AND STUDENT GUIDE



Carolina BioKits™ Wright Readi-Stain®

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TEACHER'S MANUAL

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STUDENT GUIDE*

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*Photocopy the Student Guide as needed for use in your classroom.

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NOTES

Overview

In this activity, students learn how to stain prepared human blood smears. Students observe the different types of cells present in human blood. Then, they answer assessment questions and make drawings of cells based on their observations. The activity requires 50 minutes and is designed to accommodate 30 students working in pairs.

Objectives

Students will

- stain and observe human blood cells.
- identify the erythrocytes and lymphocytes present in blood.

Content Standards

To view the national and local standards met by this kit, visit www.carolina.com/correlations.

Time Requirements

Preparation20 minutes

Activity50 minutes

Materials

Included in the kit:

- 2 dropper bottles of Wright stain
- 2 dropper bottles of Wright stain buffer
- 30 human blood smear slides
- 2 human blood smear slides stained with Wright stain
- 15 Human Blood Smear cards
- Teacher's Manual and reproducible Student Guide

Needed, but not supplied:

- 15 compound microscopes (oil immersion with 100× objective, if available)
- oil for oil immersion slide preparation (if applicable)
- distilled water
- bibulous paper
- 15 plastic plates or trays
- 15 timers

NOTES

Safety

Use this kit only in accordance with established laboratory safety practices, including appropriate personal protective equipment (PPE). Ensure that students understand and adhere to these practices. Know and follow all school district guidelines for the disposal of laboratory wastes.

Wright stain can stain skin and clothing. Have students wear gloves and goggles when handling the stain, buffer, and slides, and have them wash their hands thoroughly after using these materials.

All human blood smear slides from Carolina Biological use blood from identifiable, healthy donors. Smears are fixed in an alcohol solution that also acts as a disinfectant. Because they have been fixed, the slides are not considered potentially infectious materials under the OSHA Bloodborne Pathogens standard (29 CFR 1910.1030).

Background

In humans, erythrocytes (red blood cells) are produced by the red marrow of long bones such as the femur and humerus, as well as in ribs and vertebrae. The primary role of erythrocytes is to carry oxygen throughout the body. The life span of a red blood cell can range from 100 to 120 days. Early in its development, a red blood cell has a large nucleus and appears to have no pigment. As the cell approaches maturity, it gets smaller and loses its nucleus. Hemoglobin, the protein to which oxygen binds, is formed on the surface of the cell. The mature erythrocyte is then released into the bloodstream via a special connective tissue network in the red bone marrow. The concentration of erythrocytes in blood ranges from 5 to 5.5 million cells per milliliter.

Anemia is an abnormal condition characterized by low hemoglobin levels. An iron-deficient diet can cause anemia, as can extensive blood loss. Another type of anemia, sickle cell anemia, is caused by a genetic disorder that results in the production of abnormal hemoglobin. The important consequence in each case is that the tissues are deprived of oxygen, resulting in effects ranging from minor discomfort to death.

Leukocytes (white blood cells) exist at a concentration of approximately 8000 per milliliter of blood. This quantity may fluctuate considerably with emotional state, exercise, and other variables. A primary function of leukocytes is to protect and defend the body against disease. The uncontrollable proliferation and release of leukocytes from the red bone marrow can cause a condition known as leukemia, a form of cancer of the white blood cells. Leukemia is accompanied by anemia, because the production of erythrocytes is reduced as the red bone marrow cells are displaced by leukocytes.

Oil Immersion Technique

Special objective lenses are designed for oil immersion. Before you place oil on the slide, be certain that the lens on your microscope is an oil immersion lens. Most oil immersion lenses are 100×, giving a total magnification of 1000×. To observe a slide using oil immersion, place a drop of immersion oil on the slide.

NAME _____

DATE _____

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Background

In this activity, you will stain and observe a smear of human blood. Microscopic examination of the smear reveals the cell types found in human blood.

Blood is constantly flowing through your body, for the most part, unnoticed. When you exercise, you can feel your heart pounding, working harder to pump blood that delivers oxygen to the cells of your muscles. The veins in your arms and hands have a bluish color, but if you have ever had a paper cut, you have seen the dark red color of fresh blood. What are the components of blood that give it such characteristic color?

Erythrocytes

Erythrocytes (red blood cells) make up 44% of the total volume of blood, and more than 99% of the cells in blood. To put the size of these cells in perspective, one drop of blood contains 5 million erythrocytes, and the average human body has approximately 25 trillion red blood cells! Red blood cells are produced within the marrow of long bones (e.g., the femur and humerus) at a rate of 2 million cells per second. As erythrocytes mature, they lose their nuclei and their mitochondria. Erythrocytes are biconcave disks; this shape gives the red blood cells a larger surface area and helps them better fulfill their primary role, which is to carry oxygen to every cell in the body. Specifically, oxygen molecules bind to hemoglobin, a specialized protein on the surface of red blood cells. Hemoglobin contains iron, which gives red blood cells (and blood) their characteristic dark red color. Potentially, each red blood cell can transport more than a billion oxygen molecules. Red blood cells have short life spans, living only 100–120 days. At the end of their life span, erythrocytes are destroyed in the liver and spleen.

Leukocytes

Leukocytes (white blood cells) are produced in the bone marrow, like red blood cells. Typically, between 4,000 and 11,000 leukocytes can be found in a single drop of blood. However, these numbers increase during an immune response to infection. The primary function of leukocytes is to protect and defend the body against invasion and disease. While erythrocytes are confined to the body's circulatory system, leukocytes are more commonly found outside the blood, patrolling interstitial fluids and the lymphatic system. Unlike erythrocytes, leukocytes do possess nuclei and mitochondria, and have the potential to live from months to years. They are larger than red blood cells and may even move in an amoeboid fashion.

There are two classes of leukocytes: granulocytes and agranulocytes. Granulocytes are characterized by their namesake granules, which are observable as small dots in the cytoplasm. These granules actually are vesicles that contain powerful digestive enzymes. Different types of granulocytes contain different enzymes and trigger different immune responses. Granulocytes can be recognized by their irregularly segmented, lobed nuclei. In contrast, agranulocytes lack granules and are typified by a regularly shaped nucleus. There are three types of granulocytes and two types of agranulocytes.

Granulocytes

Neutrophils exhibit phagocytic behavior by engulfing and digesting bacteria and other invading particles. They are commonly found at the site of an injury. Dead neutrophils can be recognized as the pus that forms at sites of infection. These are the most common type of white blood cell, accounting for 60–70% of

the leukocytes found in the body. The granules in neutrophilic cytoplasm turn bright pink when exposed to Wright stain. Neutrophils possess a segmented or lobed nucleus. These cells tend to self-destruct as they destroy foreign substances.

Eosinophils defend the body against larger invaders such as worms and other parasites; they are also active during allergic reactions. Only 1.5% of white blood cells are eosinophils. Their nuclei are bilobed and well defined; when stained, large red granules may be visible in the cytoplasm of the cell.

Basophils typically are produced when an injury occurs. When these cells are activated, histamine is released, promoting excess blood flow to the injured area, which consequently swells. An allergic reaction may occur when excess histamine is produced. Basophilic nuclei may have 2 or 3 lobes and may appear S-shaped. When treated with Wright stain, the large granules of basophils stain a shade of blue or violet.

Agranulocytes

Lymphocytes specialize as either B cells or T cells. B cells possess antigen receptors that bind to a particular foreign protein (called an antigen) and then signal for its destruction. An antigen might be a virus, bacterium, or parasite, or any other substance identified as foreign. T cells kill diseased cells and cancer cells. Lymphocytes account for 25–30% of all white blood cells.

Monocytes are also phagocytes. These specialized white blood cells take on amoeboid forms that engulf and destroy invading microbes. The largest blood cells, monocytes have long life spans. Approximately 5% of white blood cells in the body are monocytes.

Thrombocytes

Thrombocytes (platelets) are small, irregularly shaped cell fragments formed from large, multinucleate cells in the bone marrow. Thrombocytes serve a crucial purpose; they are responsible for releasing clotting factor proteins. Thrombocytes lack nuclei and have a life span of only about 7 days. One drop of blood contains between 250,000 and 500,000 thrombocytes.

Pre-laboratory Questions

1. What are the five types of leukocytes?
2. Which cell type is the most numerous? Estimate the percentage of each cell type present in human blood.

Materials

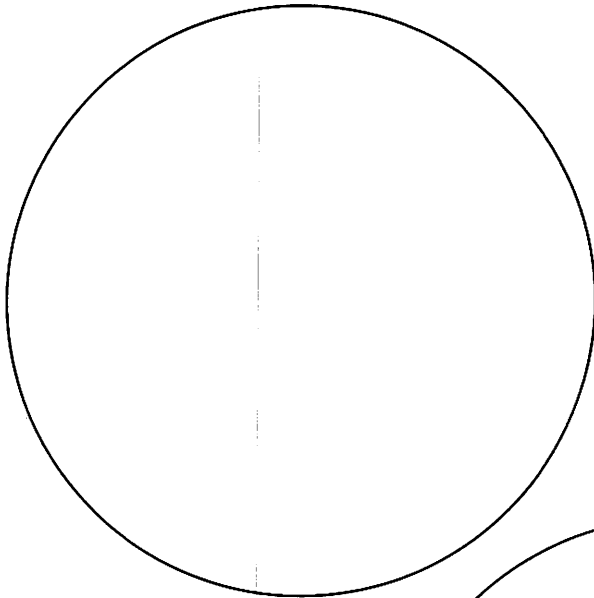
Wright stain
Wright stain buffer
human blood smear slide
plastic plate or tray
timer
distilled water
bibulous paper
compound microscope
oil for oil immersion slide preparation (if applicable)
Human Blood Smear card

Procedure

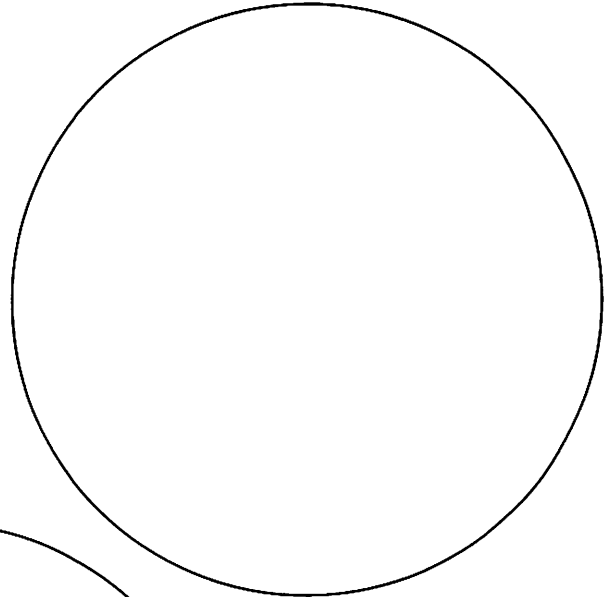
1. Obtain a prepared blood smear slide.
2. Place the slide on the plastic plate or tray.
3. Squeeze 9 drops of Wright stain onto the smear, and then let it stand undisturbed for 3 minutes.
4. Add 9 drops of buffer to the stain. Blow gently on the liquid to help mix the stain and buffer. Allow the slide to sit for 90 seconds.
5. Tilt the slide so the buffer drains off the end. Repeat Step 4. This time, allow the slide to sit for 8 minutes.
6. Gently flush the slide with distilled water.
7. After most of the water has drained from the slide, dry the slide completely by blotting it using bibulous paper.
8. After it has dried, examine the blood smear slide under a microscope according to your teacher's instructions. Use the objective lens specified by your instructor. A coverslip may not be required.
9. Use the Human Blood Smear card to help you identify the leukocytes.

Laboratory Questions

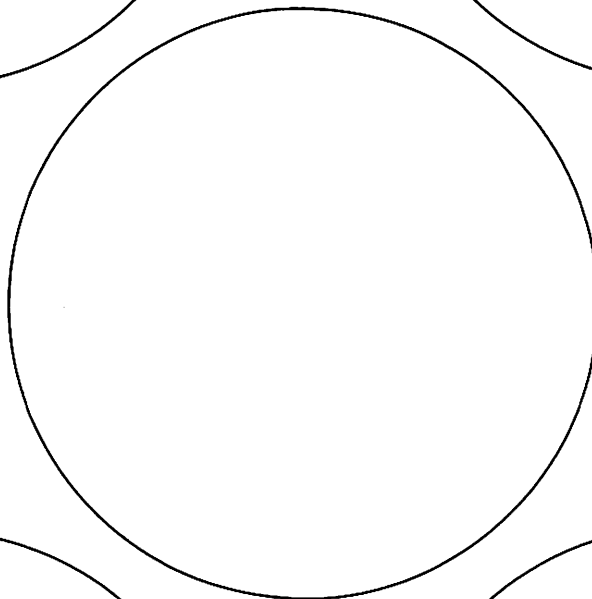
1. Try to find each of the five types of leukocytes (white blood cells) on your blood smear. Draw a picture of each type of cell you find. If you cannot locate one type, check your partner's slide. If you do not observe a cell type on either slide, note the missing type in your answer.



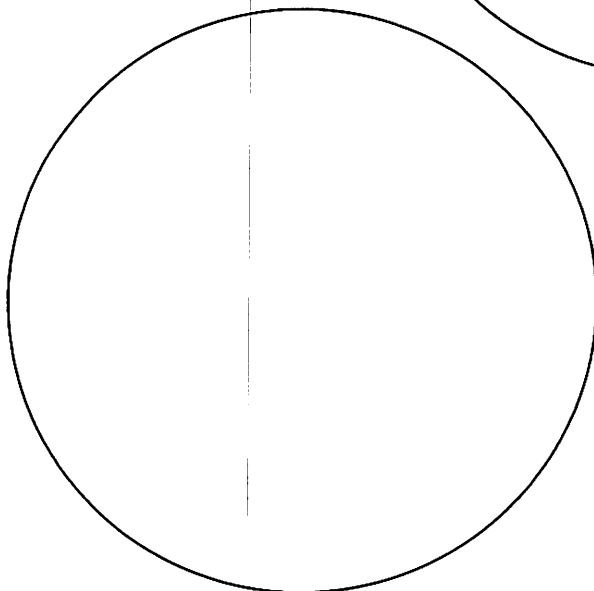
Neutrophil



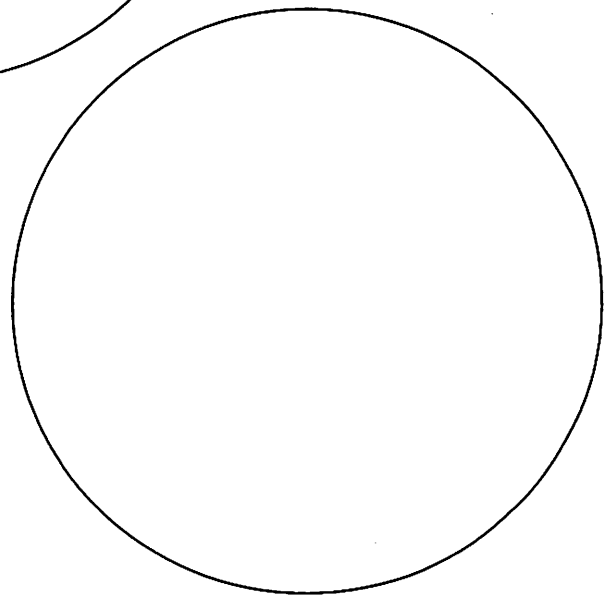
Eosinophil



Basophil



Lymphocyte



Monocyte

2. What color is the stain when it is added to the slide? What color is it after the buffer is added?

3. Describe the structure and function of erythrocytes.

4. What is the major function of leukocytes?

5. Which of the leukocytes are characterized as granulocytic? Describe the function of each of these cells.

6. Define "phagocyte." Which leukocytes act as phagocytes?



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700380**

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