
**Laboratory Activities to Enhance the Study of Whole
Blood Components
and their Role in the Immune Response**

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Teacher Guide

I. Overview

The goal of this unit is to provide students with experiences that will enhance their understanding of the concepts related to whole blood components and the relationship that these components have to the immune response. These unit materials would best be used to support instruction in a biology course during the study of immunity or possibly the circulatory system. As students progress through the unit activities they will have an opportunity to observe whole blood (porcine-pig) and isolates of neutrophils, lymphocytes and monocytes by performing a Wright Giemsa Stain. Using a microscope students will observe, count and sketch these blood components. As a follow up activity, students will again isolate viable neutrophils from fresh porcine blood and directly observe these neutrophils phagocytizing foreign yeast cells. In addition to improving student s understanding of immune\circulatory concepts, participation in this activity will provide students with memorable insight into the dynamic nature of living cells. They will be directly involved in observing diverse cell types, as well as participating in a live observation of one of the most dramatic methods of membrane transport, phagocytic endocytosis.

II. Science Background

Whole blood contains a wide variety of cell types and fragments suspended in liquid plasma. The formed elements of blood include: red blood cells (RBCs), white blood cells (WBCs) and platelets. RBCs or erythrocytes make up about 99.9% of all cellular components in whole blood. The function of whole blood is as diverse as the cell types that it contains. For example, blood components transport dissolved gases, nutrients, wastes, regulates pH, restricts fluid loss, stabilizes body temperature and notably defends against pathogen invasion. WBCs or leukocytes can be subdivided into 5 categories: neutrophils, eosinophils, basophils, monocytes and lymphocytes. These cells all play vital roles in both innate and acquired immune responses. (see Table A below).

Table A - Blood Cell Types

| Cell Name | Function | Characteristics |
|-------------------------|--|---|
| Red Blood Cell | Carries oxygen gas from the lungs to the tissues. Carries carbon dioxide gas from tissues to the lungs. | The most abundant blood cell (99.9% of total blood cells). Somewhat small and disc shaped. Contains a red pigment (hemoglobin) that binds and carries gases. |
| White Blood Cell | Defends the body against invasion by pathogens. | Composed of the 5 cell subtypes listed below. |
| Neutrophil | An early WBC to arrive at a site of infection. Attacks and destroys bacteria through phagocytic digestion. | The most numerous WBC (70% of all WBCs). Survives about 1 day in circulation. Produced in the bone marrow. Nucleus is multi-lobed. |
| Eosinophil | Attacks antibody labeled foreign materials/microbes. Can destroy by enzyme release or phagocytosis. | Nucleus is bilobed and cell contains numerous granules. Slightly larger than a neutrophil. Produced in the bone marrow. |
| Basophil | Enters damaged tissues and releases histamine to enhance inflammation. | Cell contains numerous granules. Slightly smaller than a neutrophil. Produced in the bone marrow. Very rare in circulation. |
| Monocyte | Enters tissues to phagocytize pathogens. Secretes chemicals that can attract neutrophils and initiate scar tissue formation. | Nucleus is large and often kidney shape or oval. Larger than a neutrophil. Can survive for months in circulation. Produced in the bone marrow and lymphatic tissue. |

| | | |
|-----------------|---|---|
| Lymphocyte | Involved in the attack of foreign cells. Can secrete antibodies to target specific foreign invaders. Can maintain long term surveillance for specific foreign invaders and abnormal tissue cells. | The second most numerous WBC (20% of all WBCs). Nucleus is relatively large. The smallest of the WBCs. Survives for decades in circulation. Produced in bone marrow and the lymphatic tissue. Includes subtypes such as T cells, B cells and natural killers. |
| Platelet | Initiates the clotting process and helps repair injured blood vessels. | A small cell fragment shed from a much larger bone marrow cell. Remain in circulation for about 10 days. Average number per ul of blood = 350,000 |

Each of the WBC types has specific characteristics observable with staining and magnification (see Table A above). Neutrophils are phagocytic and account for 50-70% of all WBCs. They are an essential component of a non-specific (innate) immune response. They have a unique multi-lobed nucleus and are about 5-10 um in size. Neutrophils migrate to microbe affected tissues where they attach, engulfed the microbe by pseudopodia and digested them with lysozymes. Migration to these sites of infection is often guided by positive chemotaxis, a chemical stimuli released by other WBCs or by the waste products of the microbe itself. These guardians of the immune system often represent the first line of defense against infection. Due to their short lives (1-2 days) and vital role they play in immune response, 7 million neutrophils are produced in the bone marrow every minute. This innate response is in contrast to acquired immune responses that typically involve antibody-antigen interactions. Antibodies are immunoglobulin molecules (i.e. IgG) that are produced by the stimulation of a B cell by an antigen (i.e. microbe). B Cells can produce millions of very specific antibodies for each antigen encountered. These antibodies may offer future protection against the microbe for an indefinite period of time. Antibodies protect against infection in several ways. They can interlock and disable the toxin produced by the antigen or simply coat the microbe (opsonization) thus targeting it for destruction by phagocytic WBCs. Frequently, antigen-antibody combinations initiate the production of compliment. The compliment system is a complex reaction involving more than 20 proteins. These proteins can assemble to form membrane channels in the invading microbe. These open channels then permit osmotic forces to swell and burst invading microorganisms.

Pig blood is used in this unit s laboratory exercises since it poses less health threat than human blood and it is easier to obtain. Pig blood also represents an appropriate alternative for human whole blood due to its similarity to human blood composition and immune response. Pig and Humans homologies are a result of the close evolutionary kinship of these two mammals. This

has lead to numerous medical applications including xenografts. A common pig-human xenograft example is pig skin grafting in burn patients. Similarities at the molecular level are also being established. To date, over 2000 genes have been determined to be the shared by both species. Bovine (cow) blood is not a recommended substitute due to the potential that cows might be infected with the known human pathogen, Brucilosis. All blood products should be handled with great care. Appropriate use of barriers and disinfection must be followed when using any blood product.

III. Student Outcomes

Students will have an opportunity to directly participate in activities that will give them opportunities to prepare a and observe blood slides of the neutrophils, lymphocytes and monocytes found in whole blood. In addition to these techniques students will have an opportunity to observe viable neutrophils phagocytizing foreign yeast cells. These activities will enhance students understanding of blood components and the role that they play in the immune response. Numerous basic biology concepts such as membrane transport and cytolysis will also be reinforced in the activity.

IV. Learning Objectives

Students will:

- reinforce their understanding of acquired and innate immunity.
- reinforce their understanding cell mediated and humoral response.
- reinforce their understanding of the interactions between antigen and antibody.
- identify 4 general components and functions of whole blood.
- identify 5 types of white blood cells.
- briefly describe the immune related function of each of the 5 types of white blood cells.
- describe significant characteristics of the 5 types of white blood cells.
- prepare stained whole blood smears for microscopic examination.
- isolate neutrophils (and optionally lymphocytes and monocytes) from whole blood.
- observe neutrophil phagocytosis of (foreign) yeast cells.

V. Time Requirements

The recommended period of time for the completion of this lesson is approximately 1 week. Instruction could occur over five 50 minute class periods or alternatively over three 90 minute class periods. The isolation of neutrophils and lymphocytes is time consuming and would best be implemented in a 90 minute laboratory period. Of course, advance preparation of blood samples by the teacher could allow for this isolation activity to take place in a traditional 50 minute period.

VI. Advance Preparation \ Materials and Equipment

Materials and Equipment List for a class of 24 students (4 students per lab station)

Safety Equipment

- 24 pairs of gloves
- 24 goggles
- 24 lab coats
- 6 dispensers of antibacterial soap
- 6 spray bottles of bleach 5% (50 ml of household bleach, add water to make 1.0 L)
- 1 biohazard bag for autoclave

Part 1 - Preparation of a Wright Giemsa Stained Slide Using Whole Pig Blood

- 250 ml Wright Giemsa stain
- 1.0 L distilled water
- 6 upright staining jar with lid
- 6 microscope slide and cover Slip
- 6 microscope x400 (x1000 oil immersion if available)

Part 2 - Isolation and Staining of Neutrophils from Fresh Whole Pig Blood

- 30 ml of fresh porcine (pig) blood collected in a screw cap tube containing 1ml of 1000u/ml heparin on ice (obtained and collected fresh from a local USDA inspected slaughter house)

Teacher aliquoted materials for 1 lab station(4 student group):

- 500 ul of fresh, heparinized pig blood on ice
- 500 ul of 3% Dextran-0.9% NaCl solution (3ml of Dextran, add 97ml of 0.9 % NaCl solution to make 100 ml of solution)
- 350ul of 0.9% NaCl (0.9 gm NaCl, add 99.1 gm distilled water to make 100 gm of solution)
- 200ul 1.7% NaCl (1.7 gm NaCl, add 98.3 gm distilled water to make 100 gm of solution)
- 100ul Hypaque-Ficoll
- 200 ul distilled water
- 20 ul of PBS buffer
- 10 disposable 1ml pipettes
- 4 microcentrifuge tubes, 2ml
- 1 microcentrifuge variable speed 1400-1800 rpm
- 250 ml Wright Giemsa stain
- 1.0 L distilled water
- 6 upright staining jar with lid
- 6 microscope slide and cover Slip
- 6 microscope x400 (x1000 oil immersion if available)

Part 3 - Microscopic Observation of Neutrophil Phagocytosis of Bakers Yeast

- 20 ul of neutrophils recently isolated from fresh porcine blood and resuspended in PBS

- 100 ml of Bakers Yeast solution (Mix 1.0 g sugar into 100 ml of warm water and add 0.1 g of dry active Bakers Yeast. Let stand for 1 hr prior to use.)
- 1 microscope slide and cover slip
- microscope x400 (x1000 oil immersion if available)

Safety Issues

- As with any blood product, appropriate barriers, disinfectants and universal precautions must be used in the laboratory. Students and teachers should be gloved and wear protective goggles and lab coats.
- All blood product contaminated solutions should be disposed of in a waste jar containing a 50% household bleach solution. After 1 hr of bleach the waste solution may be poured down the drain by the teacher and the container placed in the biohazard bag for autoclaving.
- All disposable plasticware should be autoclaved appropriately as potential biohazards.
- All working surfaces and non-disposable equipment should be disinfected. Hand washing is mandatory for all participants at the end of these labs.
- The blood sample must be obtained fresh, immediately placed on ice and used within 24 hrs.

Additional Notes

- * Solution prep will require about 1 hr to complete.
- * Pig blood must be collected fresh, heparinized, iced and used within 24hrs. (A USDA inspected slaughter house will likely be the most convenient source of blood. A local veterinarian might also be able to provide you with a small quantity of fresh mammal blood.)
- * Phagocytosis will be more vigorous if the pig blood is used soon as soon as possible and no later than 24 hours after collection.
- * Inform students that the centrifuge load must be balanced.
- * Consumable materials such as Sodium Heparin, Wright Giemsa stain, Dextran, Hypaque-Ficoll and PBS buffer may be ordered through the Sigma catalog of Biochemicals and Reagents (For general information call, 800-521-8956.)
- * Cost of the specialized consumable materials in this lab should run no more than \$20.00 for a class of 24 students. The first time purchasing expenses of these consumables should not exceed \$80.00. A number of these substances may be obtained as donations from local hospital and universities laboratories.

VII. Student Prior Knowledge

All blood products present some potential risk of pathogen transmission and students should be very familiar and strictly adhere to Universal Precautions. Students must be well versed in and

appreciate the importance of safe laboratory practices before they may participate in this lab. For example, students recognize the importance of constant barrier protection using gloves, goggles and lab coat. They should be familiar with the proper method of removal and disposal of gloves, be able to identify materials that should be placed in the biohazard bag and the liquid waste bleaching jar, disinfection of lab counter tops and proper hand washing techniques.

Prior to participating in this unit students are expected to be familiar with the basic concepts of innate and acquired immunity. Students should also have a fundamental understanding of the general blood components and their function.

Prior laboratory skills should include: microscope use, basic slide preparation, pipette use, centrifuge use.

VIII. What Is Expected From The Students

Upon completion of this unit, students will be expected to have receive an 80% or better on a test that covers concepts related to the components of blood, their general morphology and function. Particular emphasis will be placed on the specific roles that leukocytes (WBCs) play in immunity. A general understanding of innate and acquired immunity will also be assessed.

Participation in the lab activities is also expected. Students will perform a Wright Giemsa stain of whole porcine blood, isolate neutrophils from whole porcine blood and observe the phagocytosis of foreign yeast cells by these neutrophils. Concepts and laboratory skills will be assessed by requiring students to answer pre and post laboratory questions. A series of formally labeled microscope sketches will also be completed. Required sketches will include: porcine whole blood components and neutrophil phagocytosis of foreign yeast.

As an extension activity students may isolate lymphocytes from the buffy interface between the Hypaque Ficoll layer and saline solution. The application of a Wright Giemsa stain to these cells will enhance the microscopic observation of these neutrophil and lymphocyte isolates. Students may wish to sketch and label the contrasting characteristics of these two distinctly different WBCs.

IX. Anticipated Results

Observation of whole porcine blood should be very similar to human blood in terms of the percent composition and general morphology. As in humans, porcine whole blood should be dominated by erythrocytes (RBCs). Students will very likely have to scan across the slide in order to see the less common WBCs and platelets. See Photo 1 for an example of Wright Giemsa stained WBCs.



Photo 1 - The more numerous cells with multi-lobed nuclei are neutrophils. The dark, large cell with numerous granules and a bi-lobed nucleus is an eosinophil. The smaller cell with a very large nucleus is a lymphocyte.

The isolation of porcine neutrophils should yield large numbers of viable, mobile, phagocytic cells. They should be visible at x400 without staining. With the addition of a small quantity of yeast phagocytosis can be observed at x400 under the microscope within seconds. Observations of these cells will be improved if a x1000 oil immersion microscopes was available.

Troubleshooting

Unsuccessful or low quality observations of phagocytosis in this lab might be related to several factors. The most common problem will likely be related to not obtaining and using fresh whole blood within 24 hours. Additionally, students may have difficulty following the correct sequence of the procedure steps. Students may also damage cells with too vigorous of pipetting when resuspending cell pellets. Aliquoting of isolation reagents by the teacher greatly decreases the possibility of student measurement error and is strongly encouraged.

X. Classroom Discussion

The use of blood in the classroom and the related safety considerations is an opportune time to discuss the general health concerns related to body fluids, infectious disease and barrier protection. Stress that students should treat all body fluids cautiously as a potentially infectious substance. To place additional emphasis on the issue distribute a zip lock bag with a pair of vinyl gloves to each student. Encourage them to place it in the glove box of their car for emergencies that they might encounter. This will help to set the tone for a safe laboratory experience. Be sure to stress that noncompliance with safety issues will result in their immediate removal from the lab.

Ask students to consider analogies for the function of blood cell types. For example, basophils are like firemen who enter a burning building (inflammation) and spray water (histamine) on the fire.

Be sure to discuss the related general biology concepts as they are encountered in the labs.

Osmosis plays a large role in the isolation of neutrophils. Isotonic solutions are maintained until distilled water is used to lyse the RBCs. Another concept that should be stressed relates to the phagocytosis of yeast. This is a dramatic example of cell membrane transport and a good opportunity to review the principles of active transport. When students observe the movement of neutrophils this is a great opportunity to reinforce the idea that cells are not the flat, static objects portrayed on the pages of their text books but just the contrary. Cells are three dimensional and dynamic. Remarkably, the movement of neutrophils is driven by the same protein found in our muscles - actin.

XI. Assessment

After a formal presentation of the concepts related to immunology and blood cell types (see II. Science Background), some type of formal test\quiz should be administered to assess the conceptual readiness of students prior to the lab activities. Before beginning the lab students should be presented with general laboratory concepts, guidelines related to safety and equipment use. In particular, blood precautions, proper centrifuge use and rationale for the key procedure steps of the lab should be covered. Again, a test\quiz is strongly recommended to assess student understanding. Any students that are unable to demonstrate a mastery of the safety related concepts and requirements should not be permitted to participate in these lab activities.

References

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Voyich, Jovanka and DeLeo, Frank. Host-pathogen interactions: leukocyte phagocytosis and associated sequelae. Methods in Cell Science 2002: 24: 79-90.

Student Section

I. Rationale - Overview

Your body is a frequent target of abuse. Minor scrapes, cuts, burns and exposure to actively infectious people all occur frequently and provide opportunities for bacteria and viruses to invade and exploit your body. These events can set the stage for potentially life threatening infections. In these laboratory exercises you will explore ways that your body can fight back against these threats. Your body's defense system is known as the immune response and certain components of your blood working in coordination with the lymphatic system play a major role in how it works. In order to better understand the immune response we will explore its close interactions with blood.

Whole blood contains a wide variety of cell types and fragments suspended in liquid plasma. The formed elements of blood include: red blood cells (RBCs), white blood cells (WBCs) and platelets. RBCs or erythrocytes make up about 99.9% of all cellular components in whole blood. The function of whole blood is as diverse as the cell types that it contains. For example, blood transports dissolved gases, nutrients, wastes, regulates pH, restricts fluid loss, stabilizes body temperature and notably defends against pathogen invasion. WBCs or leukocytes can be subdivided into 5 categories: neutrophils, eosinophils, basophils, monocytes and lymphocytes. These cells all play vital roles in both innate and acquired immune responses. (see Table A below).

Table A - Blood Cell Types

| Cell Name | Function | Characteristics |
|-------------------------|---|--|
| Red Blood Cell | Carries oxygen gas from the lungs to the tissues. Carries carbon dioxide gas from tissues to the lungs. | The most abundant blood cell (99.9% of total blood cells). Somewhat small and disc shaped. Contains a red pigment (hemoglobin) that binds and carries gases. |
| White Blood Cell | Defends the body against invasion by pathogens. | Composed of the 5 cell subtypes listed below. |
| Neutrophil | The first WBC to arrive at a site of infection. Attacks and destroys bacteria through phagocytic digestion. | The most numerous WBC (70% of all WBCs). Survives about 1 day in circulation. Produced in the bone marrow. Nucleus is multi-lobed. |

| | | |
|-----------------|---|---|
| Eosinophil | Attacks antibody labeled foreign materials/microbes. Can destroy by enzyme release or phagocytosis. | Nucleus is bilobed and cell contains numerous granules. Slightly larger than a neutrophil. Produced in the bone marrow. |
| Basophil | Enters damaged tissues and releases histamine to enhance inflammation. | Cell contains numerous granules. Slightly smaller than a neutrophil. Produced in the bone marrow. Very rare in circulation. |
| Monocyte | Enters tissues to phagocytize pathogens. Secretes chemicals that can attract neutrophils and initiate scar tissue formation. | Nucleus is large and often kidney shape or oval. Larger than a neutrophil. Can survive for months in circulation. Produced in the bone marrow and lymphatic tissue. |
| Lymphocyte | Involved in the attack of foreign cells. Can secrete antibodies to target specific foreign invaders. Can maintain long term surveillance for specific foreign invaders and abnormal tissue cells. | The second most numerous WBC (20% of all WBCs). Nucleus is relatively large. The smallest of the WBCs. Survives for decades in circulation. Produced in bone marrow and the lymphatic tissue. Includes subtypes such as T cells, B cells and natural killers. |
| Platelet | Initiates the clotting process and helps repair injured blood vessels. | A small cell fragment shed from a much larger bone marrow cell. Remain in circulation for about 10 days. Average number per ul of blood = 350,000 |

Each of the WBC types has specific characteristics observable with staining and magnification (see Table A above). Neutrophils are phagocytic and account for 50-70% of all WBCs. They are an essential component of a non-specific (innate) immune response. They have a unique multilobed nucleus and are about 5-10 um in size. Neutrophils migrate to microbe affected tissues where they attach, engulf the microbe by pseudopodia and digest them with lysozymes. Migration to these sites of infection is often guided by positive chemotaxis , a chemical stimuli

released by other WBCs or by the waste products of the microbe itself. These guardians of the immune system often represent one of the first line of defenses against infection. Due to their short lives (1-2 days) and vital role they play in immune response, 7 million neutrophils are produced in the bone marrow every minute. This innate response is in contrast to acquired immune responses that typically involve antibody-antigen interactions. Antibodies are immunoglobulin molecules (i.e., IgG) that are produced by the stimulation of a B cell by an antigen (i.e., microbe). B Cells can produce millions of very specific antibodies for each antigen encountered. These antibodies may offer future protection against the microbe for an indefinite period of time. Antibodies protect against infection in several ways. They can interlock and disable the toxin produced by the antigen or simply coat the microbe (opsonization) thus targeting it for destruction by phagocytic WBCs. Frequently, antigen-antibody combinations initiate the production of complement. The complement system is a complex reaction involving more than 20 proteins. These proteins can assemble to form membrane channels in the invading microbe. These open channels then permit osmotic forces to swell and burst invading microorganisms.

During this laboratory exercise you will be examining the cellular components of fresh blood to better understand the immune response. Due to the safety concerns that surround the use of human blood, pig blood is used as a safer alternative. Pig blood is similar to human blood and therefore can be used as a model for our exploration. Pig and Humans homologies are a result of the close evolutionary kinship of these two mammals. This has led to numerous medical applications including xenografts. A common pig-human xenograft example is pig skin grafting in burn patients. Similarities at the molecular level are also being established. To date, over 2000 genes have been determined to be shared by both species. The lab has three major parts:

Part 1 - Preparation of a Wright Giemsa Stained Slide Using Whole Pig Blood

Following the preparation of the slide you will microscopically view the types and characteristics of the cellular components of whole blood. Estimated blood cell counts will be made.

Part 2 - Isolation and Staining of Neutrophils from Fresh Whole Pig Blood

This isolation procedure relies on a combination of chemical separation techniques using Dextran to precipitate RBCs and Hypaque-Ficoll as a gradient (filter) to isolate neutrophils from the other RBCs and other WBCs. Isolation is also enhanced by using centrifugation and distilled water to promote the osmotic lysis of RBCs. Wright Geimsa stains of neutrophils (and optionally of lymphocytes and monocytes) may be performed at this step.

Part 3 - Microscopic Observation of Neutrophil Phagocytosis of Baker s Yeast

Active Neutrophils will engulf the foreign Bakers yeast. Bakers yeast is used as a non-pathogenic model to simulate an invading microbe.

II. Materials -Lab Station of 4 Students

Part 1 - Preparation of a Wright Giemsa Stained Slide Using Whole Pig Blood

- 4 pairs of gloves

- 4 goggles
- 4 lab coats
- 1 dispenser of antibacterial soap
- 1 spray bottles of bleach 5%
- 1 biohazard bag for autoclave
- 50 ml Wright Giemsa stain
- 200 ml distilled water
- 1 upright staining jar with lid
- 1 microscope slide and cover Slip
- 1 microscope x400 (x1000 oil immersion if available)

Part 2 - Isolation and Staining of Neutrophils from Fresh Whole Pig Blood

-
- 4 pairs of gloves
 - 4 goggles
 - 4 lab coats
 - 1 dispenser antibacterial soap
 - 1 spray bottles of bleach 5%
 - 1 biohazard bag for autoclave
 - 500 ul aliquot of fresh porcine (pig) blood containing 1ml of 1000u/ml heparin on ice
 - 500 ul aliquot of 3% Dextran-0.9% NaCl solution
 - 350 ul aliquot of 0.9% NaCl
 - 200 ul aliquot of 1.7% NaCl
 - 100 ul aliquot of Hypaque-Ficoll
 - 200 ul aliquot of distilled water
 - 20 ul of PBS buffer
 - 10 disposable 1ml pipettes
 - 4 microcentrifuge tubes, 2 ml
 - 1 microcentrifuge variable speed 1400-1800 rpm
 - 50 ml Wright Giemsa stain
 - 200 ml distilled water
 - 1 upright staining jar with lid
 - 1 microscope slide and cover Slip
 - 1 microscope x400 (x1000 oil immersion if available)

Part 3 - Microscopic Observation of Neutrophil Phagocytosis of Bakers Yeast

- 4 pairs of gloves
- 4 goggles
- 4 lab coats
- 1 antibacterial soap
- 1 spray bottles of bleach 5%
- 1 biohazard bag for autoclave
- 20 ul of neutrophil - PBS solution recently isolated from fresh pig blood

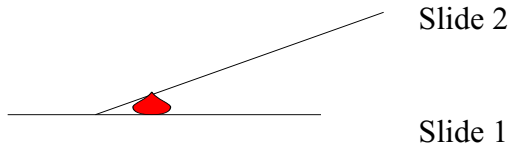
- 20 ul aliquot of baker yeast solution
- 1 microscope slide and cover slip
- microscope x400 (x1000 oil immersion if available)

III. Procedure

Part 1- Preparation of a Wright Giemsa Stained Slide Using Whole Pig Blood

Day 1

1. Wear lab coat, goggles and gloves at all times during this lab.
2. Place one drop of fresh pig blood on a slide.
3. Touch one end of a second slide at a 30 degree angle to the drop on the first slide.



4. Push the second slide along the first to create a thin smear across the first slide.
5. Let the smear air dry overnight.
6. At the conclusion of the lab dispose of all contaminated material in the biohazard bag, wipe down work station surfaces with bleach solution, dispose of gloves and wash hands.

Day 2

1. Wear lab coat, goggles and gloves at all times during this lab.
2. Place blood smear slide into a staining jar and cover with Wright Giemsa stain for 30 seconds.
3. Destain by placing the slide into the staining jar and cover with distilled water for 3 minutes.
4. Gently rinse slide with distilled water.
5. Let slide dry for 10 minutes.
6. View under x400 or if possible x1000 magnification.
7. Record your observation as Sketch 1. Label recognizable blood cell types.
8. Count cell types and record cell numbers and percentages in Table 1.
9. At the conclusion of the lab, dispose of all contaminated material in the biohazard bag, wipe down work station surfaces with bleach solution, dispose of gloves and wash hands.

Part 2 - Isolation of Neutrophils from Fresh Whole Pig Blood

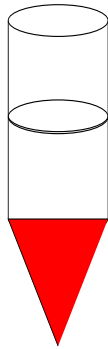
Day 1

1. Wear lab coat, goggles and gloves at all times during this lab.
2. Obtain aliquoted solutions required for this activity from your teacher. Be sure to keep the whole pig blood on ice in a Styrofoam cup until needed.
3. Use disposable 1ml pipettes to add 500 ul of whole pig blood to 500ul of the 3% dextran

solution. Cap and gently invert to mix. Let stand for 20 minutes.

4. Pipette the supernatant to a clean microcentrifuge tube. Dispose of the dark red precipitate.

Microcentrifuge Tube



Supernatant

Precipitate Dark Red Bottom Layer

5. Centrifuge at 1800 RPM for 10 minutes.

6. Pipette the supernatant and dispose into the liquid waste container.

7. Resuspend the pellet in 350 ul of 0.9% NaCl solution. *It is essential that this resuspension by pipetting be done gently to avoid lysing the cells.*

8. Use a pipette to carefully underlay 10 ul of Hypaque - Ficoll beneath the cell suspension.

9. Centrifuge at 1400 RPM for 25 minutes.

10. The buffy interface between the saline solution and the Hypaque-Ficoll contains lymphocytes and monocytes. This portion of the supernatant may be pipetted and saved for staining. The pellet contains neutrophils and must be saved. Discard supernatant in liquid waste container.

11. Gently resuspend pellet in 20ul of distilled water to selectively lyse remaining RBCs. Gently mix with pipette for 20 seconds.

12. Quickly add 20 ul of 1.7% NaCl solution.

13. Centrifuge at 1400 RPM for 10 minutes.

14. Pipette and dispose of supernatant.

15. Gently resuspend pellet in 10 ul of PBS solution.

16. Prepare a smear of the neutrophil- PBS solution and let dry overnight. (Optionally you may also prepare a smear of the lymphocytes and monocytes.)

17. If you wish to observe neutrophil phagocytosis immediately proceed to part three of the activity.

18. At the conclusion of the lab, dispose of all contaminated material in the biohazard bag, wipe down work station surfaces with bleach solution, dispose of gloves and wash hands.

Day 2

1. Wear lab coat, goggles and gloves at all times during this lab.

2. Obtain the neutrophil smear from the previous day and perform a Wright Giemsa stain.

3. At the conclusion of the lab, dispose of all contaminated material in the biohazard bag, wipe down work station surfaces with bleach solution, dispose of gloves and wash hands.

4. View under x400 or if possible x1000 magnification.
5. Record your observation as Sketch 2. Label recognizable WBC types.
6. Count cell types and record cell numbers and percentages in Table 2.
7. At the conclusion of the lab, dispose of all contaminated materials in the biohazard bag, wipe down work station surfaces with bleach solution, dispose of gloves and wash hands.

Part 3 - Microscopic Observation of Neutrophil Phagocytosis of Baker's Yeast

Day 1

1. Wear lab coat, goggles and gloves at all times during this lab.
2. Place one drop of the recently isolated neutrophil - PBS solution onto a clean slide.
3. Place one drop of the Baker's Yeast solution onto the neutrophil - PBS drop.
4. Immediately view cells at x400 (or if available x1000) magnification. Observe for signs of phagocytosis.
5. Record your observation as Sketch 3. Label recognizable cell types using Table A and Photo 1 as a reference.
6. At the conclusion of the lab, dispose of all contaminated materials in the biohazard bag, wipe down work station surfaces with bleach solution, dispose of gloves and wash hands.

Safety Notes

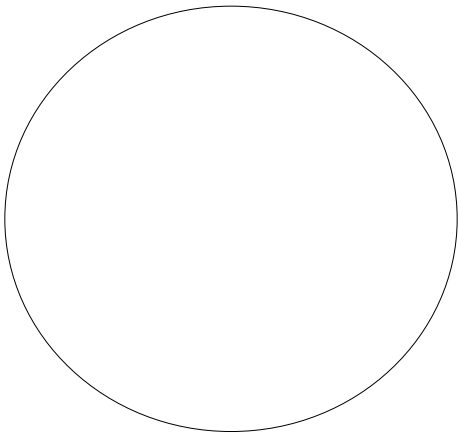
- As with any blood product, appropriate barriers and Universal Precautions must be used in the laboratory. Students should be gloved and wear protective goggles and lab coats during the entire lab activity.
- All blood contaminated solutions should be disposed of in a disposal container of 50% household bleach solution.
- All disposable plasticware should be disposed of in biohazard bags..
- All work surfaces and non-disposable equipment should be disinfected. Hand washing is mandatory for all participants at the end of these labs.
- Centrifuges must be balanced and have the lid closed when in use.

V. Data Collection

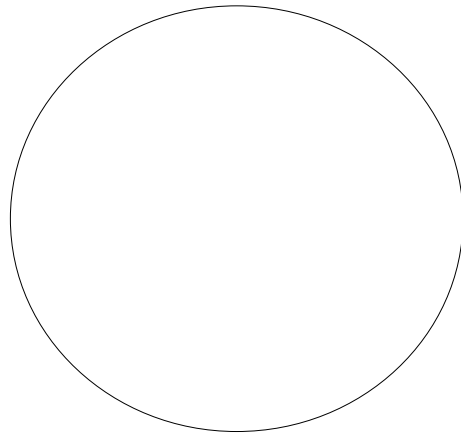
Name _____ Class _____ Date _____

Observations

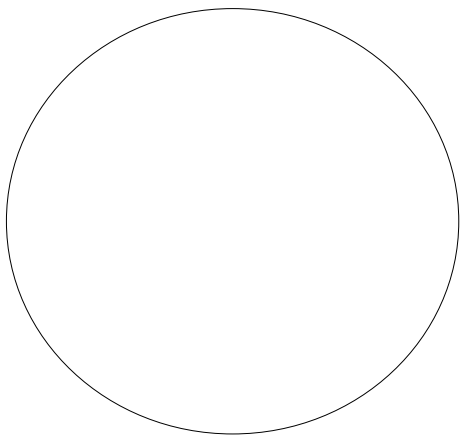
Sketch 1 - Whole Pig Blood



Sketch 2- Neutrophil Isolate



Sketch 3 - Neutrophil Phagocytosis of Yeast



Data Table 1 - Estimated Blood Cell Count in Field of View

| | Field Count | % of Field |
|-----------|-------------|------------|
| RBCs | | |
| WBCs | | |
| Platelets | | |

Data Table 2 - Estimated WBC Count in Field of View Neutrophil Isolation

| | Field Count | % of Field |
|-------------|-------------|------------|
| Neutrophils | | |
| Eosinophils | | |
| Basophils | | |
| Monocytes | | |
| Lymphocyte | | |

Analysis and Conclusion Questions

Pre-Lab Question

1. What blood cell types do you expect to be most abundant in your field of view during the observation of whole pig blood? What blood cell type do you expect to be the least abundant when you scan across your whole pig blood slide? Do you believe that the percent composition of blood cell types will be consistent with the human blood counts? Why or why not?

Post-Lab Questions

Part 1 - Preparation of a Wright Giemsa Stained Slide Using Whole Pig Blood

2. Was your hypothesis concerning the relative abundance of pig blood cell types consistent with the data collected and recorded in Table 1?

3. What could be responsible for an increase in WBC count in an individual? How might medical doctors use a WBC counts to assist in making a diagnosis with a patient experiencing stomach pain?

Part 2 - Isolation of Neutrophils from Fresh Whole Pig Blood

4. What blood cell type is being precipitated by dextran, removed and discarded in step 4? Hypothesize how dextran might cause these cells to precipitate out of solution?

5. A NaCl solution of 0.9% is considered isotonic with the internal environment of cells. This concentration of solution helps to keep the cells in osmotic homeostasis. What was the purpose of resuspending cells in distilled water (step 11)? What was the purpose of adding 1.7% NaCl 20 seconds later (step 12)?

6. Was the isolation of neutrophils successful? What evidence supports your conclusion?

7. Is there any evidence of non-neutrophil cells in the isolate? What might account for the presence of non-neutrophil blood cells in the isolate?

Part 3 - Microscopic Observation of Neutrophil Phagocytosis of Bakers

8. Why is it so important to work quickly with the isolated neutrophils during the phagocytosis activity?

9. In this activity you are observing the phagocytosis and destruction of foreign microbes (yeast). Describe the details of a possible immune response scenario that might describe this event.

10. List each specific type of blood cell observed during parts 1-3 of this lab. Briefly describe their function.

VI. Answers to Analysis and Conclusion Questions

Part 1 - Preparation of a Wright Giemsa Stained Slide Using Whole Pig Blood

1. What blood cell types do you expect to be most abundant in your field of view during the observation of whole pig blood? What blood cell type do you expect to be the least abundant when you scan across your whole pig blood slide? Do you believe that the percent composition of blood cell types will be consistent with the human blood counts? Why or why not?

RBCs account for 99.9% of all blood cells. Eosinophils, basophils and monocytes would likely be the least common cells. Due to the homologies (due to common phylogeny) found between human and pigs blood counts would likely be similar between the two species.

2. Was your hypothesis concerning the relative abundance of pig blood cell types consistent with the data collected and recorded in Table 1?

Students hypothesis will likely be supported.

3. What could be responsible for an increase in WBC count in an individual? How might medical doctors use a WBC counts to assist in making a diagnosis with a patient experiencing stomach pain?

The presence of an active pathogen would cause your body to mount an immune response that would involve an increase in the WBC count. An elevated WBC count could be used to distinguish between pain originating from something like an intestinal obstruction vs pain from an infection such as peritonitis or appendicitis.

Part 2 - Isolation of Neutrophils from Fresh Whole Pig Blood

4. What blood cell type is being precipitated by dextran, removed and discarded in step 4? Hypothesize how dextran might cause these cells to precipitate out of solution?

RBCs are the primary blood cell being removed by the Dextran. Dextran might act as a chemical sieve. The size or perhaps some other physical feature of RBCs allows them to move more freely through the dextran to the bottom of the centrifuge. Other cells having their own unique properties are left above in the supernatant.

5. A NaCl solution of 0.9% is considered isotonic with the internal environment of cells. This concentration of solution helps to keep the cells in osmotic homeostasis. What was the purpose of resuspending cells in distilled water (step 11)? What was the purpose of adding 1.7% NaCl 20 seconds later (step 12)?

Distilled water will selectively destroy any fragile RBCs that remain in the sample. Distilled water would eventually damage the WBCs so it is critical to quickly return the concentration of the solution back to approximately 0.9% by adding an equal volume of 1.7% to the 0.0% distilled water and preserve the WBCs.

6. Was the isolation of neutrophils successful? What evidence supports your conclusion?

Students should see a fairly pure isolate of neutrophils under their microscopes. This should be evident by a comparison of the whole blood slide with the isolate slide.

7. Is there any evidence of non-neutrophil cells in the isolate? What might account for the presences of non-neutrophil blood cells in the isolate?

Some slight contamination by lymphocytes may be evident. This is often due to contamination by the lymphocytes trapped in the buffy layer of the Hypaque-Ficoll. (See Day 1, Part II, Step #10)

Part 3 - Microscopic Observation of Neutrophil Phagocytosis of Bakers Yeast

8. Why is it so important to work quickly with the isolated neutrophils during the phagocytosis activity?

Neutrophils are extremely short lived. They only survive for about 24hrs.

9. In this activity you are observing neutrophil phagocytosis and destruction of foreign microbes (yeast). Describe the details of a possible immune response scenario that might describe this event.

Neutrophils are called into action as an early responder to foreign microbes. They are attracted to the waste products of the invading organism. The neutrophils use pseudopodia to crawl towards its target, envelopes it and releases toxic chemicals and digestive enzymes to destroy it. They are a critical component of the non-specific, innate immune response.

10. List each specific type of blood cell observed during parts 1-3 of this lab. Briefly describe their function.

The most commonly observed blood cells in the lab are: RBCs, neutrophils, lymphocytes and possibly eosinophils. (See Table A for details of function)

