LAB-AIDS® #2 BLOOD CLOTTING EXPERIMENT KIT

Student Worksheet and Guide

PROBLEM: How does the blood clot?

The individual is protected from excessive bleeding from minor wounds by the ability of the blood to form a clot. Clotting is essentially a chemical and physical process, although many of the details are still unknown. The process is intricate and delicately balanced.

Thromboplastin initiates the clotting process and two other materials in the blood plasma are necessary for it to function. One is calcium and the other is prothrombin. The resulting reaction is:

Thromboplastin + Calcium + Prothrombin ⟶ Thrombin

The second step in forming a clot involves the reaction between thrombin and soluble blood plasma protein, fibrinogen. The reaction may be represented as:

Fibrinogen + Thrombin ⟶ Fibrin

The fibrin threads produced form the basis of the clot. They are actually minute white strands forming a meshwork which trap the blood cells until an efficient block has formed across the opening to stop the flow of blood.

Calcium is involved in the process of clotting. Changes in the concentration of calcium dissolved in the blood therefore affect the rate of clotting. If the calcium ions in the blood are precipitated by sodium oxalate, citrates or fluorides, the blood will not clot. Those substances act as anti-coagulants.

In the following exercises you will concentrate on the visible aspects of the clotting process.

The first three exercises should be performed by a team of 3 students so that each student will only have to draw blood ***once*** and yet be able to do ***three*** blood studies.

Identify the members of the team as "A", "B", and "C". The procedure for drawing blood should be studied carefully before attempting this lab exercise.

1. Swing the non-writing hand vigorously several times.
2. Scrub the middle finger of the non-writing hand with the ***Individualized Saturated Alcohol Pad*** and allow to air dry. ***Do not contaminate*** **this finger.**
3. Using the *Individual Sterile Lancet,* puncture the tip of the finger with a firm quick stroke.
4. Wipe away the first drop of blood with the alcohol pad: This first drop is primarily serum.

A team of three students (A, B, C) will perform the following exercises (I, II, III).

EXERCISE:

I. Observing a clot
II. Determining coagulation time
III. Determining bleeding time

PROCEDURE: (Outline)

1. Student A draws his blood, studies clotting
   Student B determines A's coagulation time
   Student C determines A's bleeding time, then,
2. Student B draws his blood, studies clotting
   Student C determines B's coagulation time
   Student A determines B's bleeding time, then,
3. Student C draws his blood, studies clotting
   Student A determines C's coagulation time
   Student B determines C's bleeding time, then,

I. OBSERVING CLOTTING

1. The student drawing blood should scratch an "X" on one end of a plastic slide with a pin or other sharp object. Place the slide on a microscope stage and focus on the "X" with low power.
2. Leave the microscope in focus, but remove the slide so that a drop of blood can be placed on the surface marked with an "X".
3. The student drawing blood should do so as described above.
4. Place a drop of blood on the clean plastic slide. ***Do not cover with a cover slip.***
5. *Quickly* place the slide on the microscope stage and focus on the *odge* of the drop. Observe thin black fibrin threads forming at the edge. Be prepared to work quickly as the first threads form within seconds. The microscope has already been focused.
6. Observe for 5 minutes. Draw and describe the network of fibrin formed.

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7. Leave the slide in focus on the microscope and return periodically during the lab period. Describe the changes that take place.

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II. DETERMINING COAGULATION TIME

1. Student who has drawn blood for 1, above , should wipe the punctured finger with the alcohol pad and squeeze the finger to produce *another* drop of blood. Wipe away the first drop as it coagulates abnormally fast. As soon as the second drop is formed, the second student should hold the end of a capillary tube horizontally in it and let the tube run full of blood. When the tube is filled, *note the time* on the chart below.
2. Keep the capillary tube in a closed hand so as to keep it as close to body temperature as possible. After one minute, carefully break a small piece of the tube (about 1/4"), and *slowly* separate the two pieces. Note whether a thread of coagulated blood connects the two pieces of tubing. Repeat every 30 seconds until the coagulation time of the blood sample has been determined. Record the elapsed time in the chart below.
3. What is the average coagulation time for the class?

III. DETERMINING BLEEDING TIME

1. Student who has drawn blood for I, above, should again squeeze the punctured finger to make it bleed. When bleeding starts, note the time in the chart below.
2. Using a piece of absorbent paper, the third student should blot up the blood every 20 seconds. Do *not* touch or press the skin again. What happens to successive drops?

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3. Note the time of the last drop. Record the elapsed time in the chart below.
4. What is the average bleeding time for the class?

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Students should follow the procedure outline, with each student drawing blood.

IV. DETERMINING THE EFFECT OF CALCIUM CONCENTRATION

1. Wash off the slide used in observing a clot. At the end marked with an "X," place a drop of calcium carbonate solution. At the other end, place a drip of saline-citrane solution. Mix a drop of blood with each solution, using *separate* toothpicks. Observe both drops under the microscope. Describe the clotting response of each and explain.

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	Coagulation Time			Bleeding Time		
	Student A	Student B	Student C	Student A	Student B	Student C
Time begun						
Time ended						
Time elapsed						
Class average						

1. Briefly describe the clotting process. _____

_____

2. Compare your coagulation and bleeding times. What explanation can you offer for any differences?

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3. What evidence is there that calcium concentration affects the clotting process?

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Student's Name _____ Date _____