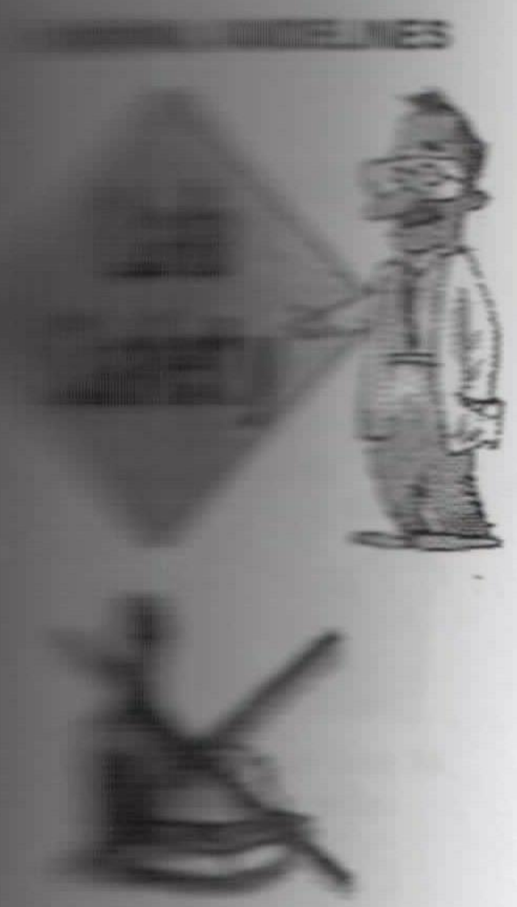
Lab Safety Rules



1. Conduct yourself in a responsible manner at all times in the laboratory.

2. Follow all written and verbal instructions carefully. If you do not understand a direction or part of a procedure, ASK YOUR TEACHER BEFORE PROCEEDING WITH THE ACTIVITY.

3. Never work alone in the laboratory. No student may work in the science classroom without the presence of the teacher.

4. When first entering a science room, do not touch any equipment, chemicals, or other materials in the laboratory area until you are instructed to do so.

5. Perform only those experiments authorized by your teacher. Carefully follow all instructions, both written and oral. Unauthorized experiments are not allowed.

6. Do not eat food, drink beverages, or chew gum in the laboratory. Do not use laboratory glassware as containers for food or beverages.

7. Be prepared for your work in the laboratory. Read all procedures thoroughly before entering the laboratory. Never fool around in the laboratory. Horseplay, practical jokes, and pranks are dangerous and prohibited.

8. Always work in a well-ventilated area.

9. Observe good housekeeping practices. Work areas should be kept clean and tidy at all times.

10. Be alert and proceed with caution at all times in the laboratory. Notify the teacher immediately of any unsafe conditions you observe.

11. Dispose of all chemical waste properly. Never mix chemicals in sink drains. Sinks are to be used only for water. Check with your teacher for disposal of chemicals and solutions.

12. Labels and equipment instructions must be read carefully before use. Set up and use the equipment as directed by your teacher.

13. Keep hands away from face, eyes, mouth, and body while using chemicals or lab equipment. Wash your hands with soap and water after performing all experiments.

14. Experiments must be personally monitored at all times. Do not wander around the room, distract other students, startle other students or interfere with the laboratory experiments of others.

15. Know the locations and operating procedures of all safety equipment including: first aid kit(s), and fire extinguisher. Know where the fire alarm and the exits are located.

16. Know what to do if there is a fire drill during a laboratory period; containers must be closed, and any electrical equipment turned off.

CLOTHING

17. Any time chemicals, heat, or glassware are used, students will wear safety goggles. NO EXCEPTIONS TO THIS RULE!

18. Contact lenses may be not be worn in the laboratory.

19. Dress properly during a laboratory activity. Long hair, dangling jewelry, and loose or baggy clothing are a hazard in the laboratory. Long hair must be tied back, and dangling jewelry and baggy clothing must be secured. Shoes must completely cover the foot. No sandals allowed on lab days.

20. A lab coat or smock should be worn during laboratory experiments.

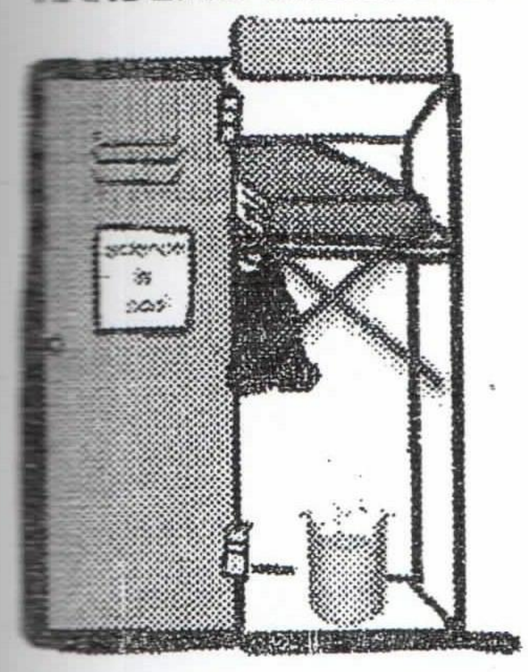
ACCIDENTS AND INJURIES

21. Report any accident (spill, breakage, etc.) or injury (cut, burn, etc.) to the teacher immediately, no matter how trivial it seems. Do not panic.

22. If you or your lab partner is hurt, immediately (and loudly) yell out the teacher's name to get the teacher's attention. Do not panic.

23. If a chemical should splash in your eye(s) or on your skin, immediately flush with running water for at least 20 minutes. Immediately (and loudly) yell out the teacher's name to get the teacher's attention.

HANDLING CHEMICALS



24. All chemicals in the laboratory are to be considered dangerous. Avoid handling chemicals with fingers. Always use a tweezer. When making an observation, keep at least 1 foot away from the specimen. Do not taste, or smell any chemicals.

25. Check the label on all chemical bottles twice before removing any of the contents. Take only as much chemical as you need.

26. Never return unused chemicals to their original container.

27. Never remove chemicals or other materials from the laboratory area.

HANDLING GLASSWARE AND EQUIPMENT

Care in handling glassware and electricity

28. Never handle broken glass with your bare hands. Use a brush and dustpan to clean up broken glass. Place broken glass in the designated glass disposal container.

29. Examine glassware before each use. Never use chipped, cracked, or dirty glassware.

30. If you do not understand how to use a piece of equipment, ASK THE TEACHER FOR HELP!

31. Do not immerse hot glassware in cold water. The glassware may shatter.

HEATING SUBSTANCES

We want to avoid this.

32. Do not operate a hot plate by yourself. Take care that hair, clothing, and hands are a safe distance from the hot plate at all times. Use of hot plate is only allowed in the presence of the teacher.

33. Heated glassware remain very hot for a long time. They should be set aside in a designated place to cool, and picked up with caution. Use tongs or heat protective gloves if necessary.

34. Never look into a container that is being heated.

35. Do not place hot apparatus directly on the laboratory desk. Always use an insulated pad. Allow plenty of time for hot apparatus to cool before touching it.

ملاحظة: مادة الامتحان تشتمل على الـSafety rules موجودة بال manual ..

WBC's & RBC's Count

- **WBC's or RBC's count**: It's the count of total number of leukocytes, or erythrocyte in a volume of blood, (WBC/1 $mm^{3)}$ blood.

- **WBCs "leukocyte":**

1- Colorless, nucleated cells
2- Formed from stem cells in bone marrow
3- Main function: Defense mechanism "Phagocytosis"
4- Normal range: Newborn (9000-30000 cell/mm^3 blood)
Adult (4000-11000 cell/mm^3 blood)

- **RBCs "erythrocyte"**:

1- No nucleus
2- Biconcave in appearance
3- Contain red pigment called hemoglobin
4- Function: to combine with O_2 and lesser extent to Co_2 and transport them through the blood vessels.
5- Life span: 120 days
6- Normal range: Male (4.5-6 million/mm^3 blood)
Female (4-5.5 million/mm^3 blood)
Newborn (up to 8 million/mm^3 blood)

- **Material used in both procedures**:

1- Blood sample (EDTA-Anticoagulant blood or capillary blood is preferred)

2- WBC's diluting pipette, have three marks (0.5, 1.0 and 11), and have a white bead.
RBC's diluting pipette, have three marks (0.5, 1.0 and 101), and have red bead.

3- Diluting fluid: For WBCs count is 2% of acetic acid with methylene blue.
For RBCs count is Hayeme's solution "Isotonic solution 50% NaCl"

4- Microscope

5- Hemacytometer: Counter chamber, used for cell counting.
- It's constructed--- so the distance between the bottom of the cover slip and the surface of the counting area is 0.1mm (1/10 mm).
- Surface of the chamber contain ---- 2 squares ruled areas (identical) separated by H- shaped moat.
- Each square has total area $9mm^2$ --- divided into nine primary squares, with an area of $1mm^2$ of each.
- Four larger corner squares ---- each one contain 16 secondary squares, and these four squares used for WBCs counting.
- Central primary square--- used for RBCs counting--- consist of 25 secondary squares, each one of 25 divided to 16 tertiary squares.

- **Procedure**: for both WBCs & RBCs count, same steps with some differences

1- With a safety bulb draw blood up to 0.5 marks on WBC's pipette complete to 11 with WBC's diluting solution, but in RBC's draw blood up to 0.5 marks on RBC's pipette and complete to 101 with RBC's diluting solution.

2- Mix for 2-3 minute, for both.

3- Charge Hemacytometer.

- Load the counting chamber with diluted blood as follow :(in both)
  1- Discard the first 4-5 drops.
  2- Place tip of the pipette at edge of the central platform of hemacytometer slide and let a drop of diluted blood run between the hemacytometer slide and cover slip by capillary.
  3- Let the hemacytometer to strand on the bench for 3-5 minute so the cells are settled down.

- **Count and Calculate**:
  1- In counting WBCs --- use magnification = 10X, BUT in counting RBCs--- use 40X.
  2- Counting: In WBCs-- - Count the number of cells in four large Squares.
  - Count the cells in each large squares starting from the upper left medium square.
  - Count all the cells within each squares, including cells touching the line at the top and on the left, BUT, cells touching the line on the right and at the bottom should not be counted.

- Also in RBCs counting, the same steps and rule, But, you count the cells in 5 medium squares, each one contain 16 (tertiary) squares---- total 80 squares.

3- Calculation: (in both procedure)
  1- Number of cells/cubic mm blood =
  Counted cell in 4 large square x dilution factor x volume correction factor

  2- The dilution factor (WBCs) = total volume/ sample volume
  = 11-1/0.5
  = **20 (dilution factor of WBCs)**

  The dilution factor (RBCs) = 101-1/0.5
  = **200 (dilution factor of RBCs)**

  3- Volume correction factor = Desired volume/ counted volume
  = $1mm^3$ / counted volume

Total volume of the four large squares (WBCs) =Volume x Number of large squares
= (Width x length x depth) x 4
= (1mm x 1mm x 1/10mm) x 4
= **0.4 mm^3**

Volume correction factor (WBCs) = 1/0.4 = 2.5

Total volume of the five medium squares (RBCs) = Volume x number of squares
= (Width x length x depth) x 80
= 1/20mm x 1/20mm x 1/10mm x80
= **1/50 mm^3**

Volume correction factor (RBCs) = 1/ (1/50) = 50

- **Source of errors:** (In both WBCs & RBCs)
  1- Flooding of chamber with excess sample
  2- Failing to count all the cell in the squares or conversely including artifacts in the count.

- **Significant of the procedure:**
  1- There are two important definition related to the leukocytes:
  - Leukocytosis ---- WBCs count > (11000 cell/mm^3)
    Causes:
    1- Body defense against "bacteria, parasite, toxin
    2- Metabolic disorder
    3- Chemical and drug poisoning
    4- Acute hemorrhage

  - Leukocytopenia ---WBCs count < (4800 cell/mm^3)
    Causes:
    1- X- ray therapy
    2- Alcoholism
    3- Antibiotic therapy
    4- Typhoid infection
    5- Measles
    6- Infectious hepatitis
    7- Tuberculosis
    8- Cirrhosis of liver

2- Disorder related to RBCs:

- Anemia ---- Decrease RBCs, under normal
- Polycethemia ------ Increase in RBCs, above normal

Two type:

1- Physiological Polycethemia, up to 8 million/mm^3

Causes:

- Age --- at birth RBCs count 8-10 million/mm^3
- High altitudes

2- Pathological Polycethemia: due to

- Primary Polycethemia – RBCs >14 million/mm^3, occur in bone marrow malignancy.

- Secondary Polycethemia ---RBCs is 8 million/mm^3

Due to:

1- Respiratory disease
2- Heart disease
3- Chronic Co_2 poisoning.