

LABORATORY ACTIVITY

Recourse Person : Annisa R. Furqaani, S.Si., M.Biomed.

Subject : The Cell

Department : Medical Biology and Histology

| A | | Sequent | |
|---|---|---|-------------|
| I | Introduction | : | 30 Minutes |
| II | Pre-test | : | 5 Minutes |
| III | Lab. Activities | : | 110 Minutes |
| IV | Post-test (if needed) | : | 5 Minutes |
| B | | Topic | |
| Date: 04 October 2019 | | | |
| | 1. Observe peripheral blood smear slide | : | 40 Minutes |
| | 2. Fill in blind poster of The Cell | : | 15 Minutes |
| | 3. Lab: Osmotic fragility test | : | 40 Minutes |
| | 4. Signal transduction | : | 15 Minutes |
| C | | Venue | |
| Biomedical Laboratory, Faculty of Medicine, Unisba, Jl. Tamansari No.22 Bandung 40116 | | | |
| D | | Equipment | |
| 1 | Blood cells | 1. Human peripheral blood smear slide 2. Light microscope | |
| 2 | Cell Structure | 1. Poster of the cell | |
| 3 | Osmotic fragility test | 1. Twenty four 12 x 75 mm test tubes. 2. Two 5 mL serologic pipets (TD), one 3 mL pipet 3. Parafilm squares 4. One heparinized normal control sample 5. One heparinized patient sample 6. 1% NaCl solution 7. Distilled Water | |
| 4 | Signal transduction | 1. Macrophage cells video | |
| E | | Pre-requisite/Pre-test | |
| Note: If the pre-test score less than 50, the student can't allow to do lab. Activities | | | |
| F | | Lab. Activities | |
| 1. The Students were divided into six groups 2. Each group do lab activities accompanied by tutor | | | |
| <p>Lab Activity 1. Observe peripheral blood smear Blood smear is the method that displays the cell types of peripheral blood to greatest advantage. See various types of cells from blood smear.</p> <ol style="list-style-type: none"> 1. Prepare the light microscope (the students must skill using a light microscope). 2. Take a blood smear slide. 3. Observe with light microscope with 1000x magnification. 4. Draw various the blood cell type (erythrocyte, leukocyte, & thrombocyte). | | | |

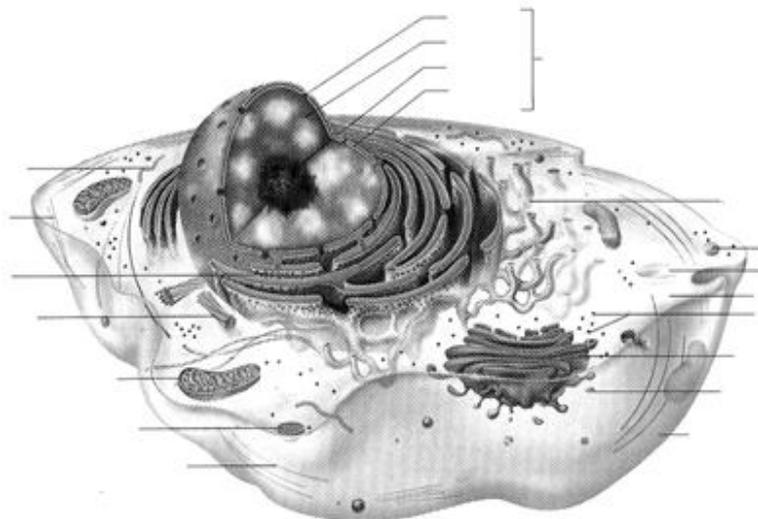
Student Task:



Lab Activity 2. Cell Structure

Students Task:

1. Fill in the cell picture!



2. Mention the function of each structure you filled in picture above.



Lab Activity 3. Osmotic Fragility Test
Principle

Whole blood is added to series of saline solutions. Exposure to hypotonic solution causes water to be drawn into the erythrocyte through osmosis. This eventually leads to swelling of the erythrocyte, leaking and bursting of the cell. Once the cell bursts, hemoglobin is released and can be measured with a spectrophotometer. The presence or absence of hemolysis is an effective measure of erythrocyte susceptibility to hypotonic damage. This the test is more than just the index of cell shape; it is also the measure of the surface to volume ratio. When an RBC's membrane surface decreases and its volume remains the same or increase, the cell becomes more turgid and less deformable. This is because of the RBC membrane is flexible but not elastic. The result of this loss of surface to volume ratio is similar to what happens to a small plastic bag that is filled with more and more water.

Spherocytes, which have a decrease surface to volume ratio, demonstrate an increased osmotic fragility. This is because of their inability to swell in a hypotonic medium before leaking hemoglobin. Sickle cells, target cells, and other poikilocytes are relatively sensitive to osmotic change therefore demonstrate a decreased osmotic fragility.

Students Task:**Procedure:**

1. Arrange 12 tubes in the rack. Label both sets of tubes 1 through 12
2. Fill each tube with 1% NaCl solution and then add with distilled water into according to the following scheme:

| TUBE | 1% NaCl (mL) | Distilled Water | NaCl % |
|------|--------------|-----------------|--------|
| 1 | 4.25 | 0.75 | 0.85 |
| 2 | 3.50 | 1.50 | 0.70 |
| 3 | 3.25 | 1.75 | 0.65 |
| 4 | 3.00 | 2.00 | 0.60 |
| 5 | 2.75 | 2.25 | 0.55 |
| 6 | 2.50 | 2.50 | 0.50 |
| 7 | 2.25 | 2.75 | 0.45 |
| 8 | 2.00 | 3.00 | 0.40 |
| 9 | 1.75 | 3.25 | 0.35 |
| 10 | 1.50 | 3.50 | 0.30 |
| 11 | 1.25 | 3.75 | 0.25 |
| 12 | 0.75 | 4.25 | 0.15 |

3. Thoroughly mixed the content of each tube by covering with parafilm and inverting several time.
4. Draw blood into tube containing heparin.
5. Immediately add 50 μ L of blood into each tube. The blood should drop directly into the solution. Do not allow the blood to drop onto the sides of the tube.
6. Incubate the tubes at room temperature for half an hour.
7. Mix gently and centrifuge at 2000 rpm for 5 minutes.
8. The result: note which tubes show initial and complete hemolysis.
 - a. Initial hemolysis is recognized by a faintly pink supernatant and a cell button at the bottom of the tube.

- b. Complete hemolysis is seen as a red supernatant with possibly a button of cell stroma at the bottom of the tube.

Interpretation:

Examples of initial and complete hemolysis in various conditions follow:

| | Initial Hemolysis (% NaCl) | Complete Hemolysis (%NaCl) |
|-----------------------------------|-----------------------------------|-----------------------------------|
| Normal | 0.45 | 0.35 |
| Hereditary spherocytosis | 0.65 | 0.45 |
| Acquired hemolytic anemia | 0.50 | 0.40 |
| Hemolytic Disease of the new born | 0.55 | 0.40 |
| Thalasemia | 0.35 | 0.20 |
| Sickle cell anemia | 0.35 | 0.20 |

Comment:

1. Fresh heparin red blood is recommended.
2. Perform this test immediately, because cell shape and osmotic conditions change with time.
3. Osmotic fragility can be altered by pH and temperature.
4. If the plasma is significantly jaundice, replace the plasma with isotonic saline before testing to prevent interference.
5. Hemolytic organism in a blood specimen can cause erroneous results owing to hemolysis, which is not attributed to test conditions.
6. If the patient has a low hemoglobin level, wash the patient and control cells once with isotonic saline and resuspend with equal volumes of RBC and saline for both specimens. This will correct for the anemia.
7. In some anemia's, when poikilocytosis accompanies a low hemoglobin level, decreased osmotic fragility maybe seen. This maybe the result partly of decreased hemoglobin concentration and not of the presence of poikilocytes.

Lab Activity 4. Signal Transduction

In responding to almost any type of stimulus the cells can detect the same percent change in a signal over a very wide range of stimulus intensities. The chemical signaling respond to *changes* the signaling ligand. Adaptation to chemical signals can occur in various ways. It results from a gradual decrease in the number of specific cell-surface receptor proteins, or rapid inactivation of such receptors. In still other cases it is due to change in the proteins involved in transducing the signal following receptor activation.

Signal-transduction cascades mediate the sensing and processing of stimuli. These molecular circuits detect, amplify, and integrate diverse external signals to generate responses such as changes in enzyme activity, gene expression, or ion-channel activity, for example signal transduction on macrophage phagocytose old red blood cell.

Students task:

Show the video about macrophages cell

1. Why macrophage cell recognize the old red blood cells?

| | |
|----------|--|
| | <p>2. Why macrophage cell can make pseudopodia?</p> |
| G | <p>References</p> <ol style="list-style-type: none"> 1. Goodman SR. Medical Cell Biology. 2nd ed. Page 1-4, 27-34, 67-102, 111-119, 195-217.1998. 2. Kumar V, Abbas AK, Fansto N, Robbin & Contran. Pathologic Basis of Dease. 7th ed. 1999; Page 625-627. 3. Harmening DM. Clinical Hematology and Fundamentals of Hemostasis. 4th ed. Philadelphia: FA Davis Co; 2002. 4. Alberts B, Johnson A, Lewis J, Morgan D, Raff, M, et al. Molecular Biology of the Cell. 6th ed. New York: Garland Science; 2017. 5. Karp G. Celullar and Molecular Biology Concepts and Experiment. 7th ed. USA: John Willey and Sons, Inc.; 2013. |
| H | <p>Home Work</p> <ol style="list-style-type: none"> 1. What is the cell? Can the cells show with naked eyes? 2. What equipment do you need to show the cells? 3. Describe architecture the cells deal with light microscope. 4. Draw the cell deal with light microscope? 5. Mention type of blood cells. 6. Mention general function of cell membrane. 7. Distinguish about diffuse and osmosis in membrane permeable? 8. Describe about osmotic fragility test and mention the procedure of osmotic fragility test. |