Name: _____

The Mystery of the Crooked Cell

An investigation into the molecular basis of a blood disorder

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As a well-respected doctor, a colleague in Chicago has written you for help. He needs your help to determine the cause of a patient's symptoms. Discuss key information from the patient's description that led you to decide what type of testing should be done. You will need evidence to support your diagnosis. Therefore, it is very important to use good laboratory practice to prevent contamination of your samples and to ensure result accuracy. In your report, explain what controls were used for the test and discuss what your results mean. This information will be shared with the patient.

Please read Dr. Herrick's letter. Three potential diseases and symptoms to be considered are listed in the table below. Circle symptoms in the table that match those described in the letter.

http://www.yorku.ca/kdenning/images/sc.jpg

Dear Colleague:

The patient reports feeling well most of the time. But he also reports odd reoccurring events. For instance, one day after a short swim he became so tired that he could hardly move. He became short of breath and complained of pain in his joints and muscles, especially the arms and legs. He felt unusually weak and required bed rest lasting a few weeks. These symptoms occurred repeatedly during his youth. He also had frequent fevers and infections. The patient complained of fatigue and soreness in the joints. Upon inspection, the whites of his eyes had a yellowish tint. He complained of pain in the left abdominal area, which was tender to the touch.

A family history reveals that he has two brothers and three sisters. None of them has this condition. His uncle and his grandmother often had similar symptoms. His grandmother died a young woman. His parents do not have this condition.

Your medical opinion in understanding this disease is appreciated.

James Herrick, MD

List three (3) symptoms the patient has.

List three (3) potential diseases that could be causing the patient's symptoms.

SOLVE THE MYSTERY

You suspect that the patient may have sickle cell anemia. Normal hemoglobin and sickle hemoglobin may look identical but these proteins have different properties. **Agarose gel electrophoresis** is a technique used to separate molecules based on charge, size or shape.

Gel electrophoresis can be used to distinguish normal hemoglobin from sickle hemoglobin based on properties of charge. Differences in the net negative charge of the hemoglobin molecules results in different rates of migration. Normal hemoglobin has a net charge of -2 and sickle hemoglobin a net charge of -1. The charge difference makes the sickle hemoglobin move differently through a matrix when an electric field is applied. By comparing the resulting movement of normal hemoglobin to sickle hemoglobin on the gel, you can distinguish between the two hemoglobin proteins.

MATERIALS

Patient Sample	Micropipette and tips
Normal Hemoglobin	Electrophoresis equipment
Sickle Hemoglobin	Microcentrifuge
Electrophoresis Buffer	Gloves

PART ONE: AGAROSE GEL PREPARATION FOR PROTEIN ELECTROPHORESIS

You will be performing protein electrophoresis on your patient and control samples. These results will be analyzed using agarose gel electrophoresis, paying specific attention to the unique banding pattern for each suspect based on fragment number and approximate size (number of bases). Part one has been done for you. The following steps are used to make the agarose gel preparation.

- ☐ 1. Locate the large glass test tube. This test tube contains 0.4 g of agarose powder and 50 ml of Tris-Glycine (TG) buffer.
- ☐ 2. Sit the large glass test tube in the boiling water bath. Occasionally stir the melting agarose to ensure even melting and a homogenous mixture.
- ☐ 3. Once all of the agarose is melted and there is no more visible powder notify an instructor to confirm that melting is complete. Give the mixture one more stir and remove the tube from the water bath and let cool for 2-3 minutes in rack on lab bench.

Pour the Gel

- ☐ 4. Carefully pour the melted agarose into the tray in the gel electrophoresis box, avoiding bubbles. If there are bubbles, use the purple comb or a pipette tip to remove them or push them to one side.
- ☐ 5. Insert the purple comb into the tray to form the wells. The purple comb has two sides - one side has wider teeth. Look at the comb and be sure the "1.5" is right side up and place the comb in a slot closest to one end of the gel (if two combs are being used on one gel, place the second comb in the center slot).
- ☐ 6. Allow the gel to solidify before moving. It takes 15-20 minutes for the gel to solidify.

How did you make the wells (holes) in the gel?

PART TWO: SAMPLE PREPARATION & PROTEIN ELECTROPHORESIS

- ☐ 7. Locate the following samples in your colored tube rack. Your instructor will assign you a gel box and a set of well numbers. Write your well numbers in the table:

Sample	What is the label on the tube?	Well number
Normal Hemoglobin		
Sickle Hemoglobin		
Patient Hemoglobin		

- ☐ 8. Centrifuge samples for two (2) seconds to collect the contents at the bottom of the tube. Make sure you balance your centrifuge!

QUICK CHECK: Compare the hemoglobin samples to each other. Can you tell a difference between them?

SAFETY FIRST! Good Laboratory Practice prevents contamination. Wear your gloves at all times, respect the agarose gel, your equipment and your other classmates.

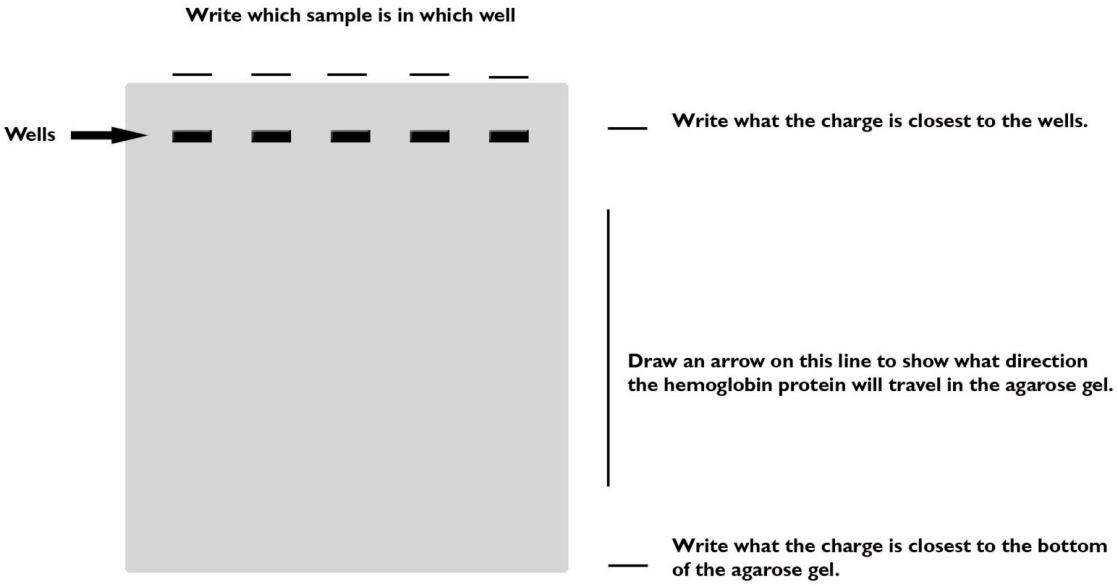
☐ 9. Load 15μL of each sample into the appropriate wells. Be sure to keep track of which wells were used and to fill out the chart above. Notify an instructor when you have finished loading your gel. They will instruct you on how to finish setting up the electrophoresis box.

☐ 10. Locate the beaker labeled "electrophoresis buffer." Slowly pour enough of the buffer into the bottom chamber of the electrophoresis box until the liquid flows over the gel and fills the upper chamber. This buffer is a salt solution. The gel should be covered entirely.

☐ 11. Run the gel at 200 volts for at least 10 minutes.

Describe any changes you see in the gel while it is running:

☐ 12. Fill in the drawing below of anticipated results with your MdBioLab instructor.

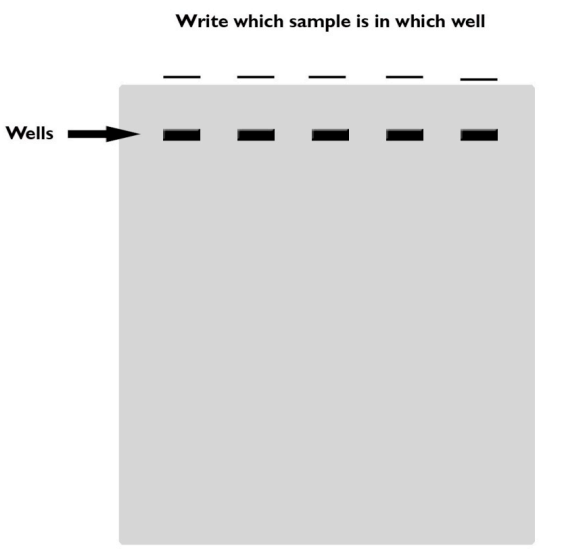


Why does the loading dye move towards the positive electrode?

What affects the rate at which the hemoglobin travels through an agarose gel?

Why did you add electrophoresis buffer to the electrophoresis box?

☐ 13. Hemoglobin is a protein that has a red color. You will be able to see how far your hemoglobin samples have migrated through the agarose gel. Draw your results below. Be sure to label which samples you drew in which wells, the direction the samples traveled, and the charge at the top and bottom of the gel:



DATA ANALYSIS

Analyze the results of your test. Observe the banding patterns on your gel. Do you see differences or similarities between the patient and the control samples?

What are the charges of normal and sickle cell hemoglobin? Which electrode on the gel box will the hemoglobin protein move towards?

Normal hemoglobin (charge): _____

Sickle hemoglobin (charge): _____

Which electrode on the gel box did the hemoglobin protein move towards (circle one)? Black (-) or Red (+)

Is your patient homozygous or heterozygous for normal hemoglobin? What about the sickle cell trait?

CONCLUSION

Think about what conclusions you can make from the experiment. When making a conclusion, scientists have to interpret the results of the test. You have compared the migration pattern of normal and sickle hemoglobin samples to that of the patient. Based on the results of your experiment, what is your diagnosis for the patient?

☐ My patient has sickle cell disease

☐ My patient does not have sickle cell disease

Draw a Punnett Square (use the diagram on the right) for your patient showing the genotypes of his parents (remember that neither parent has sickle cell disease) and circle the genotype the patient has.

Explain to the patient how you determined their test results. Assume the patient is not familiar with the test, so you'll need to describe how gel electrophoresis works (write you answer on the back of this sheet or attach an additional sheet with your explanation for the patient)