

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. A patient has come in with a multitude of symptoms and Dr. James Herrick, the man's physician, needs assistance in developing a diagnosis. Below is a letter from Dr. Herrick outlining the patient's symptoms and his family's medical history. It is up to you develop a diagnosis and then confirm it using analytical testing.

To begin your investigation, please read Dr. Herrick's letter and circle any symptoms you may feel are relevant to a proper diagnosis.



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

Dear Colleague:

While the patient reports feeling well most of the time he does experience certain odd recurring events. One day, after a short swim, he became so tired that he could hardly move. He described being short of breath and complained of pain in his joints and muscles in his arms and legs. He felt unusually weak and was put on bed rest for several weeks. These symptoms occurred repeatedly during his youth and would often result in frequent fevers and infections. The patient often complains of fatigue and soreness. Upon inspection, the whites of his eyes had a yellowish tint and his left abdominal area was tender.

A family history reveals that both his uncle and grandmother have had similar symptoms. His younger sister, an athlete who does not have similar symptoms, was once admitted to the hospital and treated for severe dehydration and joint pain after extremely strenuous exercise. Neither of the patient's parents have these symptoms.

Your medical opinion in determining this patient's diagnosis 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, a disease that affects a patient's hemoglobin. To the naked eye, normal hemoglobin and sickle hemoglobin look identical. However, 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 migration pattern of each protein. The following steps are used to make the agarose gel preparation.

### Pour the Gel

- 1. Locate the clear electrophoresis tray and white comb on the laboratory bench.
- 2. Insert the white comb into the tray to form the wells. There are two different slots in the tray for the comb. Be sure to use the slot closest to the end of the tray.
- 3. Remove the cap from the agarose gel tube and pour its entire contents into the tray. Allow the gel 15-20 minutes to solidify.

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

## PART TWO: SAMPLE PREPARATION & PROTEIN ELECTROPHORESIS

- 4. Locate the following samples in your colored tube rack and, along with the instructor, fill in the following diagram:

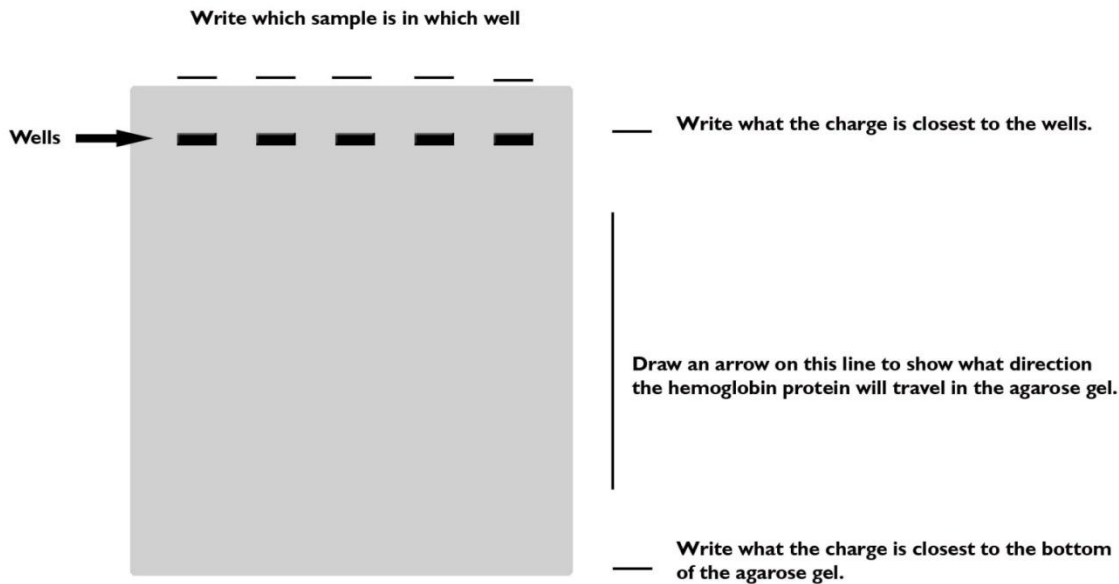
Sample	What is the label on the tube?	Well number
Normal Hemoglobin		
Sickle Hemoglobin		
Patient Hemoglobin		

- 5. Centrifuge each sample 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?

- 6. Load 15 $\mu$ L of each sample into the well that it was assigned to in the chart above.
- 7. Locate the electrophoresis box and place your sample into one of the six gel slots. The gel should be completely covered by the electrophoresis buffer. Place the lid on the box ensuring that the black wire is on top.
- 8. Run the gel at 220 volts for at least 10 minutes.

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



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

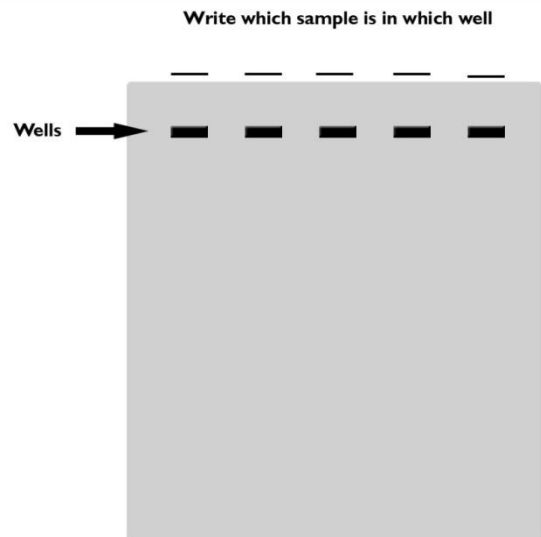
**Why did you add electrophoresis buffer to the electrophoresis box?**

**How will you use your patient's results to determine if they have sickle cell disease?**

**Since neither of the patient's parents have sickle cell disease is it possible that the patient could have it?**

**Support your theory with a Punnett square:**

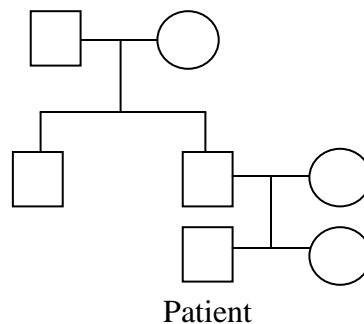
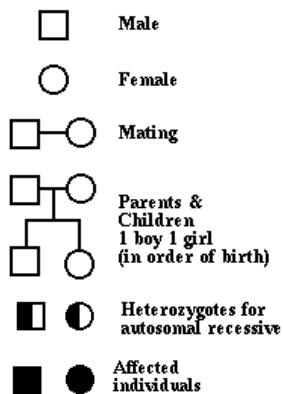

13. Draw your results below. Be sure to label which samples you drew in which wells, the direction the samples traveled, and the charge at the top and bottom of the gel:



- My patient has sickle cell disease  
 My patient does not have sickle cell disease

## DATA ANALYSIS

Genetic counselors use pedigree charts to show the occurrence and appearance of genotypes and phenotypes within a family. Recall the patient's family history from the letter on page 1 and his genotype from the analysis of his hemoglobin protein; using the pedigree below attempt to trace the occurrence of sickle cell disease within his family.



## CONCLUSION

Explain to the patient how you determined their test results. You will need to describe how gel electrophoresis works since the patient is not familiar with the test. In addition, explain the importance of genetic counseling and decide whether it is important for the patient's spouse to be tested for sickle cell trait before they have children (write your answer on the back of this sheet or attach an additional sheet with your explanation for the patient).