

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.

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) different diagnoses and the symptoms that led you to that diagnosis.

1. _____ based on symptoms: _____
2. _____ based on symptoms: _____
3. _____ based on symptoms: _____

IDENTIFY THE PROBLEM

What is the problem you are trying to solve?

SOLVE THE MYSTERY

You suspect that the patient may have a genetic disease called 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.

The following is a list of the materials you will be using for the experiment:

| | |
|------------------------|---------------------------|
| 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 protein sample based on its charge. The gel mixture has been prepared for you.

Pour the Gel

- 1. Carefully pour the melted agarose into the gel tray, avoiding bubbles. If there are bubbles, use the white comb or a pipette tip to remove them or push them to one side.
- 2. Insert the white comb into the tray to form the wells. The white comb has two sides - one side has wider teeth. Look at the comb and be sure the wider side is right side up and place the comb in a slot closest to the top of the gel tray.
- 3. Allow the gel to solidify before moving. It takes 15-20 minutes for the gel to solidify.

PART TWO: SAMPLE PREPARATION & PROTEIN ELECTROPHORESIS

- 4. 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:

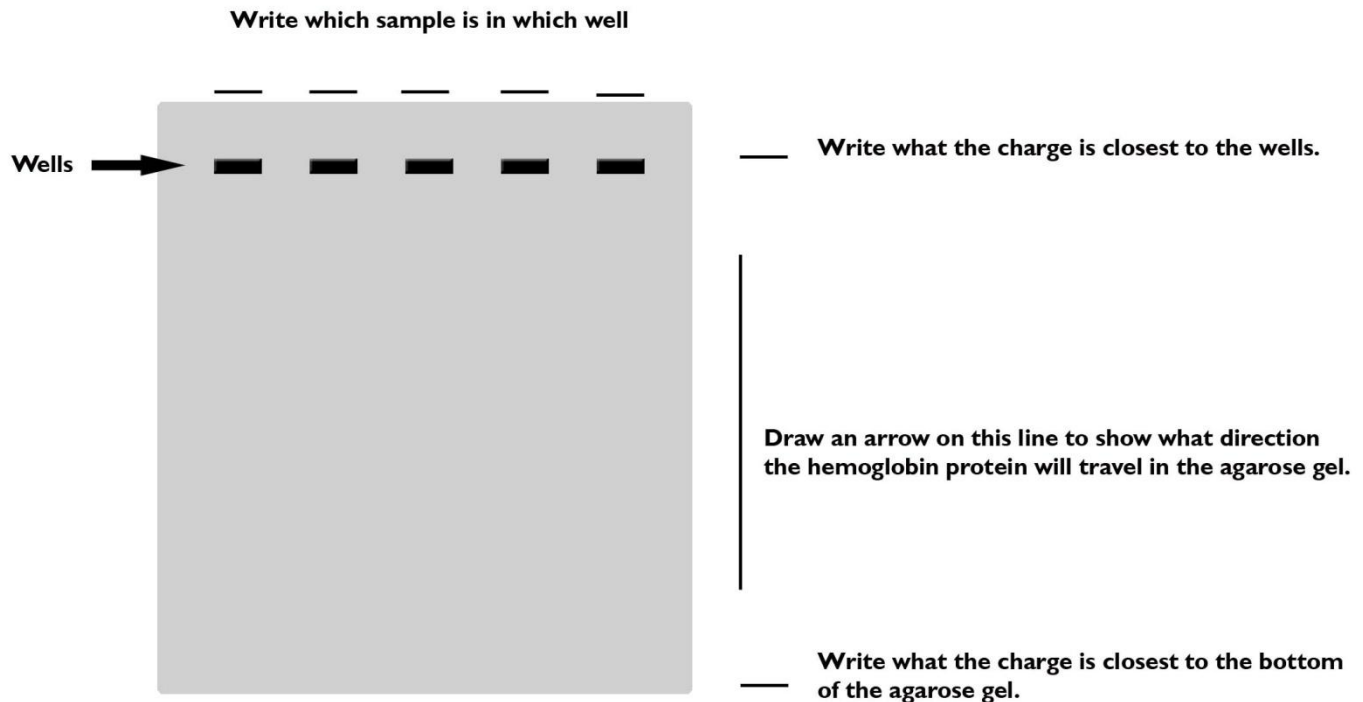
| Sample | Sample Identification Code | Well Number |
|--------------------|----------------------------|-------------|
| Normal Hemoglobin | | |
| Sickle Hemoglobin | | |
| Patient Hemoglobin | | |

- 5. 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.

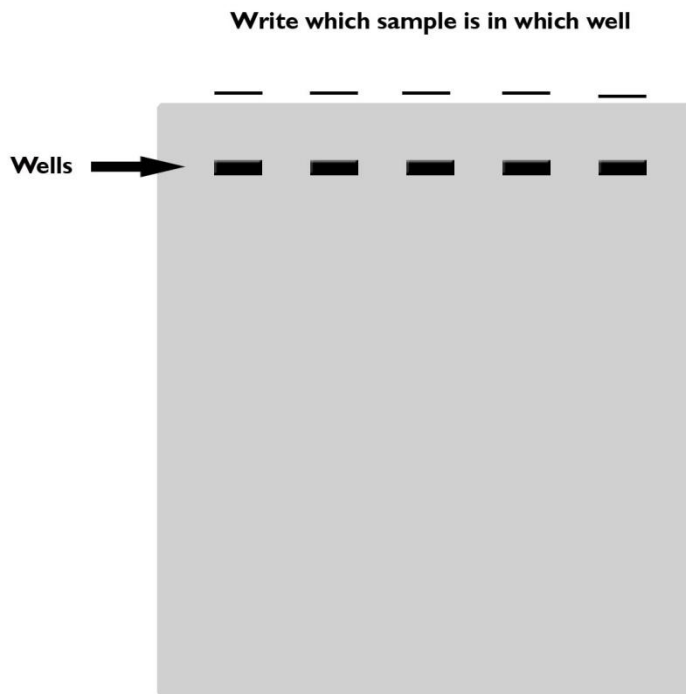
- 6. Load 20 μ L of each sample into the appropriate wells. Be sure to keep track of which wells were used and to fill out the chart in step 5. Remove the black rubber stoppers from the gel tray. Notify an instructor when you have finished loading your gel. They will instruct you on how to finish setting up the electrophoresis box.
- 7. 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. Do not pour buffer directly onto the gels. This buffer is a salt solution. The gel should be covered entirely.
- 8. Run the gel between 220 and 230 volts for at least 10 minutes.
- 9. Fill in the drawing below of anticipated results with your MdBioLab instructor.



ELECTROPHORESIS QUESTIONS:

1. What is the purpose of the agarose gel? _____
 2. What is the comb used for? _____
 3. Why did you add electrophoresis buffer? _____
-
4. What does the electricity do? _____
-
5. Which hemoglobin molecule will travel more quickly from the wells (check box)?
 - Normal hemoglobin
 - Sickle hemoglobin
 - They will travel at the same speed

10. 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 (+)

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

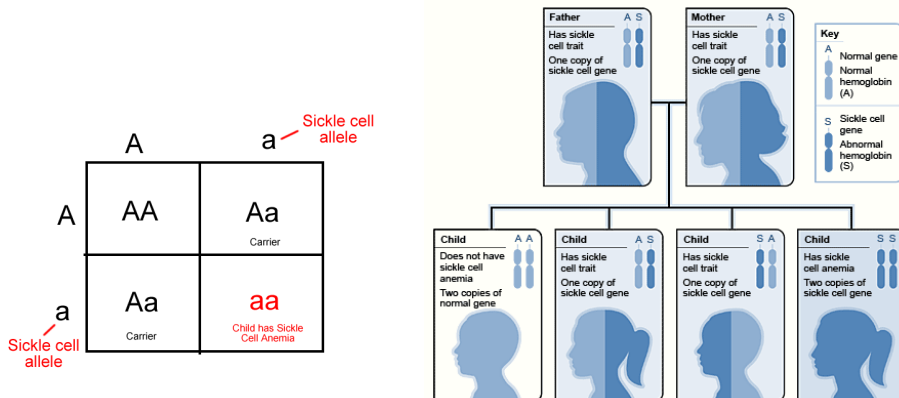
Explain to the patient how you determined their test results. Assume the patient is not familiar with the test, so you'll need to describe how gel electrophoresis works (write your answer on the back of this sheet or attach an additional sheet with your explanation for the patient)

Sickle cell anemia is a genetic disease that affects the hemoglobin protein in red blood cells. It is caused by a point mutation, also known as a single base substitution, in hemoglobin β gene. A point mutation is a change in one nucleotide base with another nucleotide base. In the case of sickle cell anemia, an A is replaced with a T converting a glutamic acid codon of GAG to a valine codon of GTG. Hemoglobin is the oxygen carrying protein in red blood cells. Red blood cells are responsible for delivering oxygen to the body.

Sickle cell anemia is an autosomal (not sex-linked) recessive disease. This means that a person must have two recessive alleles, one from each parent, to have sickle cell disease. If the patient is heterozygous (has one recessive allele and one dominant allele), the person is said to be a carrier of sickle cell trait.



<http://en.wikipedia.org/wiki/Sickle>



http://www.biologycorner.com/anatomy/blood/notes_blood_disorders.html

Normal red blood cells are round like doughnuts, and they move through small blood vessels in the body to deliver oxygen. Diseased red blood cells become hard, sticky, and shaped like sickles used to cut wheat. When these hard and pointed cells go through the small blood vessels, they clog the blood flow and break apart. This can cause pain, organ damage and a low red blood cell count or anemia.

The irregularly shaped blood cells lead to a cascade of symptoms. The sickle-shaped blood cells die prematurely, resulting in anemia and the production of excess bilirubin (a yellow pigment resulting from the breakdown of hemoglobin). Jaundice often results when the liver cannot metabolize bilirubin fast enough.

Infection, dehydration, overexertion, high altitude, chills, or cold weather can bring on a sickling episode or crisis. Sometimes there is no apparent precipitating factor. People with sickle cell anemia are susceptible to fevers and infection.

There is no cure for sickle cell anemia. Hydration, bed rest, painkillers, and antibiotics are often prescribed. Recent research has focused on re-expressing the fetal hemoglobin gene. After birth, the gene for fetal hemoglobin turns off while the gene for adult hemoglobin becomes activated. If the gene for fetal hemoglobin could be turned on again, it may compensate for the diseased hemoglobin and provide relief for people with sickle cell anemia.

Glycomimetics, a biotechnology company located in Gaithersburg, Maryland has launched a pilot study (medical trials) at Children's Hospital and Research Center Oakland in California to test GMI-1070 in sickle cell patients (1). GMI-1070 is an inhibitor that will be used to treat vaso-occlusive crisis in sickle cell patients. Vaso-occlusive crisis is the term given to the interactions of a patient's sickle cells, endothelial cells, and plasma constituents leading to complications such as pain, stroke, ulcers, and renal insufficiency(2). The study will evaluate GMI-1070's effect on the human body, blood flow, pharmacokinetics (how the body reacts to the medication from entry to exit of the body), and safety (1). Currently, patients suffering from vaso-occlusive crisis are treated with analgesics, oxygen therapy, and fluid replacement (2). GMI-1070 could provide sickle cell patients with another option of treatment.

References:

1. <http://www.glycomimetics.com/pr090902.html>
2. <http://www.aafp.org/afp/20000301/1349.html>