

the
Mystery Disease

Guided Inquiry Version of *Mystery of the
Crooked Cell* Laboratory Activity

Maryland Loaner Lab Teacher Packet

*Modified by Towson University from the
original works of Eric Howe¹ and Donald A.
DeRosa and B. Leslie Wolfe².*

www.towson.edu/fcsm/centers/stem

¹Howe, E. (2004). Using the History of Research on Sickle-Cell Anemia to Affect Preservice Teachers' Conceptions of the Nature of Science. Doctoral Dissertation, Western Michigan University, Kalamazoo, Michigan.

²DeRosa, D.A. and B.L. Wolfe. (1999). Mystery of the Crooked Cell: An Investigation and Laboratory Activity about Sickle-Cell Anemia. *The American Biology Teacher* 61 (2): 137-148.

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Maryland Loaner Lab Overview and Supplies

The Mystery Disease has two parts:

1. Pre-laboratory classroom activities that aid students in understanding the disease in terms of its mechanism of action and how histology, physiology, and genetics can all provide insight into the disease.
2. A laboratory activity that simulates a clinical test for sickle cell anemia using gel electrophoresis.

Supplied by the Maryland Loaner Lab Program (quantities listed are for 1 class set):

Description	Quantity	Comments	Must Be Returned
Teacher Packet (Binder)	1	Contains all lab information	Return
Histology Slides	2 sets	For Pre-Lab Activity B	Return
250 ml Volumetric Flask	3	For Pre-Lab Activity C	Return
100 ml Volumetric Flask	3	For Pre-Lab Activity C	Return
50 ml Volumetric Flask	3	For Pre-Lab Activity C	Return
Doughnut-shaped Clay Pieces	3 bags of 10	For Pre-Lab Activity C	Return
Sickle-shaped Clay Pieces	3 bags of 10	For Pre-Lab Activity C	Return
Differential Diagnosis Cards	5 sets of 8 cards	For Pre-Lab Activity H	Wipe clean and return
Dry Erase Markers	5	For Pre-Lab Activity H	Return
Blue-capped tubes labeled "Normal", "Abnormal", and "Unknown" Hemoglobin	1 set of 3 tubes	To develop the concept of gel electrophoresis	Return
Practice Gels	10	1 per group	Empty, clean, dry and return
Practice Loading Dye Tubes	10	1 per group	Return (with unused dye)
Gel Electrophoresis Box	1	With lid	Rinse and dry; Return
Gel Trays	6	5 trays + 1 extra	Rinse and dry; Return
Gel Combs	6	Makes 8 wells each	Rinse and dry; Return
Black Rubber Gel Tray Ends	12	2 dams per tray	Return
Power Supply	1	For use with gel electrophoresis box	Return
Graduated Cylinder (100 ml)	1	Used for pouring gels	Return
Agarose Powder Bag (0.84 g)	1 bag with tube	Enough powder to make 6 gels	Discard tube, return labeled bag
Glass Agarose Bottle (Orange Cap)	1	To make up agarose gels	Rinse and dry; Return
Micropipettes	10	1 per group, 50 µl	Return
Spatula	1	Used for moving gels	Return
Micropipette Tips	5 boxes	1 box per two groups	Return unused tips ONLY
10X TAE Buffer	1 150ml bottle	Follow Teacher Prep for dilution	Rinse and return
2 Liter Container	1	Used for mixing TAE buffer	Rinse and Return
Normal Control Samples	10 tubes ("A")	1 per group	Return unused samples
Sickle Control Samples	10 tubes ("S")	1 per group	Return unused samples
Patient (Unknown) Samples	10 tubes ("P")	1 per group	Return unused samples
Foam Microtube Racks	10	1 per group	Return
Microcentrifuge	1	Used to spin down sample tubes	Return
Disinfectant Wipes	1	Used to disinfect returned equipment	Return
Insulated Thermo-bag	1	Contains practice gels and practice dye	Return

Supplied by the Teacher:

Description	Quantity	Comments
Distilled Water	1350 ml	For making gels and running buffer
Disposable Cups	10	1 per group—to be a waste container at each workstation
*Goggles	varies	1 per student
*Gloves	varies	1 pair per student

***SAFETY: The classroom teacher must instruct students in basic laboratory safety rules and provide gloves and goggles for student use with the laboratory activity.**

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Maryland Science Core Learning Goals

Grades 9-12

Goal 1: Skills and Processes. The student will demonstrate ways of thinking and acting inherent in the practice of science. The student will use the language and instruments of science to collect, organize, interpret, calculate, and communicate information.

1. Expectation: The student will explain why curiosity, honesty, openness and skepticism are highly regarded in science.
1.1.2: The student will modify or affirm scientific ideas according to accumulated evidence.
2. Expectation: The student will pose scientific questions and suggest investigative approaches to provide answers to questions.
1.2.4: The student will test a working hypothesis. 1.2.5: The students will select appropriate instruments and materials to conduct an investigation. 1.2.6: The student will identify appropriate methods for conducting an investigation. (independent and dependent variables, proper controls, repeat trials, appropriate sample size). 1.2.7: The student will use relationships discovered in the lab to explain phenomena observed outside the laboratory. 1.2.8: The student will defend the need for verifiable data.
3. Expectation: The student will carry out scientific investigations effectively and employ instruments, systems of measurement, and materials of science appropriately.
1.3.1: The student will develop and demonstrate skills in using lab and field equipment to perform investigative techniques. 1.3.2: The student will recognize safe laboratory procedures. 1.3.3: The student will demonstrate safe handling of the chemical and materials of science. 1.3.4: The student will learn the use of new instruments and equipment by following instructions in a manual or from oral direction.
4. Expectation: The student will demonstrate that data analysis is a vital aspect of the process of scientific inquiry and communication.
1.4.2: The student will analyze data to make predictions, decisions or draw conclusions. 1.4.9: The student will use analyzed data to confirm, modify or reject a hypothesis.
5. Expectation: The student will use appropriate method for communicating in writing and orally the processes and results of scientific investigation.
1.5.1: The student will demonstrate the ability to summarize data (measurement/observations). 1.5.2: The student will explain scientific concepts and processes through drawing, writing and/or oral communication. 1.5.4: The student will use tables, graphs, and displays to support arguments and claims in both written and oral communication. 1.5.5: The student will create and/or interpret graphics.
7. Expectation: The student will show that connections exist both within various fields of science and among science and other disciplines including mathematics, social studies, fine arts and technology.
1.7.1: The student will apply the skills, processes and concepts of biology, chemistry, physics, or earth science to societal issues. 1.7.5: Students will investigate career possibilities in the various areas of science.

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Maryland Science Core Learning Goals

Goal 3: Concepts of Biology. The student will demonstrate the ability to use scientific skills and processes (Core Learning Goal 1) and major biological concepts to explain the uniqueness and interdependence of living organisms, their interactions with the environment, and the continuation of life on earth.

1. Expectation: The student will be able to explain the correlation between the structure and function of biologically important molecules and their relationship to the cell processes.
3.1.1: The student will be able to describe the unique characteristics of chemical substances and macromolecules utilized by living systems.
2. Expectation: The student will demonstrate an understanding that all organisms are composed of cells which can function independently or as part of multicellular organisms.
3.2.1: The student will explain the processes and function of related structures found in unicellular and multicellular organisms (transportation of materials, role of the circulatory system).
3. Expectation: The student will analyze how traits are inherited and passed on from one generation to another.
3.3.2: The student will illustrate and explain how expressed traits are passed from parent to offspring. 3.3.3: The student will explain how a genetic trait is determined by the code in a DNA molecule. 3.3.4: The student will interpret how the effects of DNA alteration can be beneficial or harmful to the individual, society, and/or the environment.

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Introduction

Sickle cell anemia (or sickle cell disease) is a genetic disease that affects the hemoglobin molecule in red blood cells. Hemoglobin carries the oxygen that the red blood cells deliver to all the tissues and organs of the body. Normal red blood cells (having normal hemoglobin) are round like doughnuts; they are very flexible and able to move through small blood vessels in the body to deliver oxygen. Diseased red blood cells with sickle hemoglobin become hard and are shaped like sickles used to cut wheat; they carry less oxygen to the body's tissues. When these hard and pointed cells go through small blood vessels, they can cause clots and clog blood flow. This can cause pain and tissue damage. Sickled red blood cells also do not live as long as healthy red blood cells and cause a low red blood cell count or anemia.

Each person has two copies of the gene for hemoglobin. Normal hemoglobin is referred to as hemoglobin A. The letters AA are used to indicate that both hemoglobin genes are normal. The gene that causes sickle cell anemia is referred to as hemoglobin S. There are three possible combinations of the hemoglobin A and S genes:

- AA** Individual is homozygous for the hemoglobin A gene. Both copies of the gene code for normal hemoglobin, and the person does not have the disease.
- AS** Individual is heterozygous. One copy of the gene codes for normal hemoglobin and the other copy of the gene codes for sickle cell hemoglobin. This person does not have the disease and will not develop it later in life. However, this person is considered a **carrier** of the sickle cell hemoglobin gene. Carriers are often referred to as having **sickle cell trait** because they may exhibit a few symptoms of sickle cell anemia (especially in low-oxygen environments).
- SS** Individual is homozygous for the sickle cell hemoglobin S gene. Having both copies of the gene codes for diseased hemoglobin, and this individual will suffer from sickle cell anemia.

The irregularly-shaped blood cells lead to a cascade of symptoms. The sickle-shaped red blood cells die prematurely, resulting in anemia and the production of excess bilirubin (a yellow pigment resulting from the breakdown of hemoglobin protein). Jaundice, which is the yellowing of the skin and the whites of an individual's eyes, often results when the liver cannot metabolize bilirubin fast enough.

Infection, dehydration, overexertion, high altitude, 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. Patients with sickle cell anemia will often have abdominal pain (due to the spleen trying to process all the destroyed red blood cells) and joint and muscular pain (due to blood clots).

Currently, there is no cure for sickle cell anemia. A bone marrow transplant offers a potential cure, but the procedure is risky and not always successful. There are treatments such as hydration, bed rest, painkillers, avoiding extreme temperatures, avoiding overexertion, and the use of antibiotics. Sometimes blood transfusions or even supplemental oxygen treatments are required. Recent research has focused on re-expressing the fetal hemoglobin gene as a treatment for sickle cell anemia. After birth, the gene for fetal hemoglobin turns off while the gene for adult hemoglobin becomes activated. If the gene for fetal

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hemoglobin could be turned on again, it may compensate for the diseased adult hemoglobin and provide relief for people with sickle cell anemia.

To understand the origin of sickle cell anemia, one must understand that sickled cells serve as a protective mechanism against malaria. Malaria is a deadly disease caused by a parasite transmitted by mosquitoes and found in countries along the equator. People who are carriers for sickle cell anemia (heterozygous) are protected against malaria while those with normal hemoglobin are susceptible to it. Over the years, people with the sickle cell trait (those who are carriers of sickle cell hemoglobin) migrated to other continents, which is why sickle cell anemia is seen now in areas beyond the equator. Sickle cell disease is seen often in African descendant populations but is also seen in people of other ethnic groups, including individuals from parts of the Middle East, Central India, and countries bordering the Mediterranean Sea, especially Italy and Greece.

This lesson is organized into two parts: a set of pre-laboratory classroom activities and a laboratory activity. During the pre-lab, students conduct learning activities and acquire clues about a “mystery disease” (sickle cell anemia...**but don't tell them that!**). Students will examine the mystery disease from a historical perspective; they will discover new information regarding the disease a little at a time – just like the real scientists and researchers who were studying sickle cell anemia did through the years. Each activity challenges the students to explore different aspects of the mystery disease. Students will examine how histology, physiology, and genetics provide insight into the mechanism of this mystery disease. Working in groups, students manipulate models, examine pedigrees, and gather data to construct an explanation of how the mystery disease affects the patient. Another pre-laboratory activity allows students to practice using micropipettes and practice loading agarose gels, as they can be difficult skills to initially acquire. The ***Practice Gel Loading Exercise*** instructs students regarding the proper technique used to load gels and gives them the opportunity to practice before loading the samples involved in the laboratory activity. Following the pre-lab, students participate in the laboratory activity to test a fictional patient for the presence of sickle cell hemoglobin using gel electrophoresis.

Note: Additional web resources for information on sickle cell anemia are below.

<http://www.mayoclinic.com/health/sickle-cell-anemia/DS00324>

<http://www.nhlbi.nih.gov/health/health-topics/topics/sca/>

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Pre-Laboratory Activities

Important Note

It is important NOT to tell your students the name of the disease while they are doing the lab.

The purpose of the pre-laboratory activities is to help the students explore a “mystery disease” by using a historical perspective; they will learn about the mystery disease a little at a time – just as the real scientists and researchers who studies the disease learned about it through the years. The pre-laboratory activities provide students with the opportunity to construct ideas and concepts about the mechanism of the disease.

The objectives of the pre-laboratory activities are:

- Observe normal and sickled red blood cells
- Model the movement of red blood cells through the circulatory system to gather data and make inferences about the “mystery disease”
- Analyze inheritance patterns using pedigrees
- Work cooperatively to explain the symptoms exhibited in the mystery disease
- Construct an explanation of the disease mechanism

Pre-Laboratory Materials:

Students should work in groups of 3-4 ideally; each group will need to complete activities A-D and F (Activities E, G, and H are optional). Each student will need a copy of student pages S1 - S6 and S9 - S10; however, the activity handouts should be given to the students one activity at a time.

Students can work on Activities A, B, and D-H at their desks or lab stations. For Activity C, set up each of two areas with the following materials:

- 3 empty volumetric flasks – one 50ml, one 100ml, and one 250ml
- Bag with 10 normal red blood cells (doughnut-shaped pieces of clay)
- Bag with 10 sickle-shaped red blood cells (sickle-shaped pieces of clay)

For Activity H (Differential Diagnosis), each group of students will require a set of eight laminated cards (which are provided), each with a different disease description and list of symptoms written on it.

Pre-Laboratory Engagement (10 – 15 minutes)

Organize students into groups of 3-4. Tell students that they will be playing the role of scientists (or doctors or researchers) who will be investigating a mystery disease, and they will learn about it a little bit at a time to mimic the way the real scientists learned about it through the years.

Activity A: Mystery Patient Description Sheet (10 - 15 minutes)

Students will read Dr. James Herrick’s first description of the mystery disease, which dates back to 1904. They will try to find clues as to the mechanism of the disease and decide how to proceed in their investigation of the disease.

Students should read the description of the patient who came to Dr. James Herrick, a Chicago physician, in 1904. Dr. Herrick (1861-1954) is credited with the discovery of the sickle-shaped red blood cells. Thereafter, the disease was called “sickle cell anemia” based on Dr. Herrick’s finding. **(Please refrain from mentioning the terms**

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“sickled” red blood cells or “sickle cell anemia” at this point.) The essential question is “What is the mechanism of the disease?” Working in groups, students can make observations and gather clues about the condition described in the patient scenario. Students can also identify and underline any clues in the description that may help them determine the effect of the disease on the patient.

When they are finished, invite a student from each team to write one clue on the board. Discuss the clues as a class. Ask for clarification or expansion of ideas where appropriate. Do the students think that all of the symptoms are related? If so, how? Encourage the students to think freely and make connections based on the evidence given in the patient description. The discussion usually leads to many good ideas about the mechanism of the disease. Lead the discussion towards blood and the cardiovascular system; this is especially easy if a student suggests that there might be a problem with the patient’s blood or heart. If students do not come up with this suggestion, ask a leading question, such as, “If a person experiences shortness of breath [one of the mystery patient’s symptoms], what could be wrong with them?”.

Asking students “What types of evidence would you like to help you diagnose the patient?” usually leads to at least one student mentioning examination of the blood. (If a student makes a suggestion that would require the use of modern-day equipment, such as gel electrophoresis, students should be reminded that they are doctors in the year 1904.)

Pre-Laboratory Exploration (70 – 95 minutes, not including optional activities)

Activity B: Histology Slides (15 - 20 minutes)

If microscopes are available, allow students to view the histological slides of blood smears from a person with normal blood and from the mystery patient’s blood. If microscopes are not available, you have the option of showing students a PowerPoint slide of the blood smears (<https://www.towson.edu/fcsm/centers/stem/loanerlab/>) or handing the students a worksheet with photos of the smears already on it (see student page S-22). Ideally, they will draw and describe the differences they see between the two blood smears. Students may notice the red blood cells in the mystery patient’s blood are different shapes and that there are fewer red blood cells compared with the normal patient’s blood. Also, there are sometimes more white blood cells in the mystery patient’s blood than in the normal patient’s blood.

Then, ask the students to explain how the differences might affect a person’s “circulatory health”. At least one student usually mentions that the oddly-shaped blood cells in the mystery patient’s blood might cause the blood to clot. This is a great lead-in to Activity C.

Activity C: Blood Flow Models (15 - 20 minutes)

In this activity, students are given three volumetric flasks of different sizes – 250ml, 100ml, and 50ml – that represent the arteries, veins, and capillaries (however, other combinations are possible, such as arteries, arterioles, and capillaries) and clay pieces that represent normal red blood cells (doughnut-shaped) and the mystery patient’s red blood cells (sickle-shaped). You can make this activity fairly open-ended by allowing the students to determine what the flasks represent and how to go about modeling the flow of the red blood cells through the blood vessels. Students can use differing ratios of normal to sickled blood cells. They can also vary the width of the flasks to mimic the red blood cells flowing through the different types of blood vessels.

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Students usually discover that a problem occurs when the sickled blood cells get stuck in the smallest volumetric flask forming clots in the capillaries. Discuss with the students the consequences of blood clot formation and how this new discovery ties in to the mystery patient's symptoms as described by Dr. Herrick on the Mystery Patient Description Sheet (Activity A). Also, ask the students to explain the function of red blood cells and then relate that to what is happening at the capillary-level that might be causing the mystery patient's blood cells to become oddly shaped [the red blood cells give up oxygen at the capillary level].

A good way to lead in to the next activity is to explain to the students that they have now looked at how histology and physiology play a role in helping to explain this mystery disease. What other area of biology could help us? Alternatively, ask the students what other evidence might be helpful in explaining this mystery disease.

Some **nature of science** concepts that can be incorporated into the lesson at this point are:

- 1) If not all of the groups of students come up with the same conclusions after conducting the histological examination and doing the blood flow model activity, point out to students that this is just like real science; not all scientists will come to the same conclusion when looking at the same data. Why do you suppose this is? [It is due to subjectivity, which exists in science. Scientists come from all different types of backgrounds – different cultures, different ethnicities, different socioeconomic backgrounds, different education, etc.; they all have different prior knowledge; their past experiences are not all the same. Because of these differences, scientists all bring their own biases into an investigation, so they will “see” the data differently.]
- 2) Have a discussion with the students regarding how scientists use models to help them understand certain phenomena. Scientists construct models from the observation of patterns. The models are used to represent and explain phenomena. Models are also used by scientists to make predictions about future conditions or events.

Activity D: First Pedigree Analysis (15 - 20 minutes)

For this activity, students will analyze the first of two pedigrees; the first is from 1923 and shows the prevalence of the mystery disease in the mystery patient's family. This pedigree is based on an *in-vitro* test performed by V. Emmel back in 1917.

Begin this activity by asking the students how they would determine if a particular disease or disorder is inherited (bearing in mind, of course, that they are doctors/scientists working in the year 1923).

For this activity, students should decide if the disease is inherited and, if so, what type of inheritance pattern is seen. (The most likely patterns that students may suggest are autosomal dominant, autosomal recessive, and sex-linked). Based on the inheritance pattern seen in the pedigree, the disease is autosomal dominant. (If students are uncertain, they could try doing Punnett Squares to help them determine the type of inheritance pattern seen.) Students should be able to describe the phenotypes of the following genotypes for the disease (homozygous dominant, heterozygous, and homozygous recessive) based on the inheritance pattern they chose. Ask the students to explain why the disease is not recessive or sex-linked. You could also ask the students to explain the fact that some of the mystery patient's family members had the disease but experienced much milder symptoms.

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Nature of science can also be incorporated at this point. Suppose that a group of students proposes a different way of explaining the genetics of the mystery disease. How would scientists decide which explanation is more valid? [We decide whether one explanation is better than another in many ways; one of which is by how much supportive evidence one explanation has over another. Based on this, the simple dominance theory explains the current evidence the best.]

Activity E: *In-Vitro* vs *In-Vivo* (optional; 10 – 15 minutes)

This is an optional reading and discussion activity that will allow students to discover what the terms *in-vitro* vs *in-vivo* mean and how they differ.

You can relate this new information back to the pedigree that the students analyzed in Activity D by explaining the *in-vitro* blood test Emmel used in 1917 on which the pedigree was based and asking students what were some of the problems with this test. [The main problem was that the technique placed the red blood cells under conditions that were not true-to-life: the temperature, pressure, pH, etc. were not the same as the cells in the body; and the cells were no longer receiving oxygen.]

Activity F: Second Pedigree Analysis (15 – 20 minutes)

In this activity, students will analyze a pedigree from 1947 showing the prevalence of the mystery disease in the mystery patient's family. This pedigree is based on a technique developed by I. Sherman in 1940 that more closely mimics the real-life environment to which a red blood cell would be exposed. Based on the inheritance pattern seen in this pedigree, the disease is autosomal recessive. (If students are uncertain, they could try doing Punnett Squares to help them determine the type of inheritance pattern seen.) **(Please note: sickle cell anemia is actually co-dominant as far as genotype is concerned, but it is expressed as a recessive trait because in order to develop symptoms of the disease, a person has to have both copies of the recessive sickle cell allele.)** Ask the students how the technique used to determine this pedigree differs from the one used to create the first pedigree.

Also, a very good **nature of science** question to ask the students at this point would be, “Was the hypothesis that you developed based on the 1923 pedigree wrong? Explain.” (The hypothesis regards the inheritance pattern seen in the first pedigree.) [Students should understand that their hypothesis was not wrong; it was simply a result of the information that they had at that time, which was determined by the knowledge and technology available in that time period.]

Activity G: Heterozygote Advantage (optional; 130 - 145 minutes, if done in its entirety)

In this optional activity, students will use some of the data that scientists used during the 1940s and 1950s to come up with their explanation for why an apparently deleterious recessive allele would be maintained in a population. This phenomenon is termed “polymorphism” (differing forms).

The activity begins with having the students examine the mystery disease from an ethnographical perspective. From about 1945-1954, ethnographers were trying to explain why there are certain populations in the world, such as in Uganda, Africa, where the allele frequencies for the mystery disease are quite high – and seem to be stable.

One of the ways of studying this polymorphism was by determining if a person had the mystery disease based on the results of a diagnostic blood test. The frequencies (written as percentages) on the map correspond to the frequency of carriers for the mystery disease in various parts of Uganda.

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Scientists were very surprised to find that the mystery disease was prevalent in relatively high numbers and was distributed in heterogeneous frequencies.

Explain to students that the purpose of the next part of the activity is for them to come up with explanations for why:

- a) There seems to be a high frequency of carriers of the disease in certain areas of Uganda; and
- b) There are high frequencies of carriers in some areas, while in others, there are low frequencies.

An apropos **nature of science** question that can be used at the beginning of this part of the activity is:

- As “scientists” you all have access to similar data for the Uganda Problem. Do you think that you will all come up with same explanation for the unusually high frequencies? Why or why not?

After this introduction, pass out copies of the following two handouts to each student:

1) Uganda Tribes and Allele Frequencies (page S-11):

This handout gives information about the four major ethnic (language) groups. There are three pieces of information for each group:

- a) The actual allele frequencies (as percentages) for some of the major tribes within each language group found on the map;
- b) The second column in the table, “Allele Frequency”, refers to the relative prevalence (or degree of allele frequency) that one group has with respect to the others; and
- c) The third column in the table, “Between Group Contact”, describes the amount of contact another group or groups may have had with the listed group.

Note: Each language group contained several different, distinct tribes (with much intra-language group tribal variability). However, there were distinct differences between language groups with respect to physical characteristics.

2) Uganda Tribal Group Immigration Data (page S-12):

This handout shows the four major ethnic groups (called “language groups”) that existed in Uganda. The arrows show from what direction (and how long ago) these groups colonized Uganda.

Note: This will probably be the most difficult and open-ended of the pre-laboratory activities that the students will do, so be prepared to facilitate with probing questions.

Allow time for students to work in small groups to analyze the data given and to come up with an explanation for the anomalous allele frequencies. Have student groups discuss their explanations with the rest of the class. Students should be able to back up their explanations using the evidence or inferences they made from the handouts.

At this point, refer back to the question asked earlier regarding the subjective **nature of science**: “As ‘scientists’ you all have access to similar data for the Uganda Problem. Do you think that you will all come up with same explanation for the unusually high frequencies? Why or why not?” Students often believe that if the data are examined carefully, everyone should “see” the same thing in the data; students believe that scientists are totally objective and not affected by their prior knowledge, past experiences, or their backgrounds (ethnic, cultural, educational, etc.). The intended effect of this question is to get students to consider that their explanations of the data may differ from one another due to differences in their own backgrounds that cause them to “see” the data differently.

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In the next section of this activity, students will examine how ecology (another subdiscipline of biology) can help provide insight into the mystery disease. To preface this section of the activity, state that during the time that researchers were conducting blood tests, they also noted the prevalence of certain diseases, such as malaria. Students will begin by exploring malaria in more detail.

For this part of the activity, pass out the following handout to the students:

- 1) Malaria's Vicious Cycle (page S-13): This handout depicts the life cycle of the malaria parasite. The focus of this handout is to get students thinking about the processes that are occurring at each of the stages of the disease, with an emphasis on stages 5 and 6, to see if they are able to find any correlations between the cellular or physiological aspects of malaria with what they know of the mystery disease (10 minutes).
 - You may also want students to discover the similarity between the symptoms of malaria and those of the mystery patient. (The symptoms are actually on one of the eight included Differential Diagnosis cards.)
 - Could the patient be suffering from malaria? [Students should be able to point out that malaria is a parasitic disease, while the mystery patient's disease is genetic.]
 - Also expand on the information regarding malaria, if you would like. One way to do this would be to take advantage of some of the websites and videos listed below.
 - Websites:
 - <https://www.cdc.gov/MALARIA/index.html>
 - <http://www.who.int/topics/malaria/en/>
 - Videos:
 - Deadliest Parasite on the Planet: <http://www.youtube.com/watch?v=BqjMYEfViKA>
 - HHMI – Life Cycle of Malaria Parasite in the Mosquito: http://www.youtube.com/watch?v=RqRuSwZey_U
 - HHMI – Life Cycle of Malaria Parasite in Human Host: <http://www.youtube.com/watch?v=qvITOhCmxvY>
 - Malaria: No Ordinary Mosquito Bite: <http://www.youtube.com/watch?v=IVbq2yQH52g>
 - You could also have the students work in groups of 3-4 on the following task (10-15 minutes): Students should analyze the parasite's lifecycle and come up with various ways of reducing its effectiveness as a parasite. Let students know that they should use their creativity in completing this task. If they are having problems getting started, suggest to the students to isolate each stage and think about the various mechanisms at work in each stage.

Next, in small groups, students will examine the incidence of malaria in Uganda to see if this new data can shed any light on the explanations they proposed regarding the unusually high frequencies of the allele for the mystery disease in certain areas of Uganda (30 minutes). Pass out the following two handouts:

- 1) Weather in Uganda (page S-14): This handout shows wet versus arid regions of Uganda. This handout will come into play after the students have learned about malaria. Based upon what they know about malaria, where in Uganda would malaria be most likely to occur – in arid or wet regions? Why? [In wet regions because the mosquito that transmits malaria needs water for its life cycle.]

The Mystery Disease

Pre-Laboratory Activities

- 2) Exposure to Malaria in Uganda (page S-15): This handout shows which regions of Uganda are normally malaria-free versus which experience seasonal malaria versus which regions experience continual exposure to malaria. Students should notice the relationship between the weather in Uganda and the degree of exposure to malaria. Also, if the students overlay this handout with the Uganda Tribes and Allele Frequencies handout, it will help them identify any correlations between allele frequency and the incidence of malaria (but the students should discover this on their own; see page S-16). If students do notice a correlation between the two, then challenge them to draw conclusions from this correlation.

At some point while the students are working on the above malarial data, you should interrupt them and pass out the following handout:

- 1) Anthony C. Allison's Research (page S-17): This handout describes Allison's research in which he analyzed the blood of children in Uganda to determine whether they carried the mystery disease or had a normal genotype; and whether they had the malaria parasite, including its density in red blood cells.
- This part of the activity will allow students to analyze observational data that should help them construct an alternative explanation for the high allele frequencies of the mystery disease.
 - Some background information regarding Allison's research:
 - Anthony C. Allison, MD, PhD, was interested in human polymorphisms. While conducting blood analyses, he noticed that there was a correlation between high frequencies of the allele for the mystery disease and the presence of malaria. In the regions of Uganda in which malaria was hyperendemic, there were higher relative allele frequencies of the mystery disease. Dr. Allison concluded that the mystery disease allele provided some kind of selective advantage, which was why it was not removed from the population due to natural selection.
 - Other researchers suggested that the high frequencies of the allele were due to abnormally high rates of mutation in certain geographical areas; admixture of the allele by way of intermarriage; or the disease was largely an endemically-racial phenomenon.
 - Dr. Allison did not think that the mystery disease allele was due to abnormally high rates of mutation in certain geographical areas. He did not see a reason why the mutation rate would vary by region. He also thought that in order to offset the removal of such a deleterious allele, the mutation rate would have to have been many times higher than the standard rate of mutations in animals.
 - To test his hypothesis, Allison drew blood samples from Ugandan children in an attempt to correlate the severity of malarial infection with the presence or absence of the mystery disease allele. He analyzed the blood of each child to determine whether they were a carrier for the disease or had a normal genotype, and for the presence of the malaria parasite, *Plasmodium falciparum*, including its density in red blood cells.
 - Note: There are no “-/-” patients because they would be homozygous recessive and have a high mortality rate, although some children do survive into adulthood (as the mystery patient did).
 - Allison's observational experiments supported his hypothesis (that a balanced polymorphism was evidence of protection against malaria) and it was recognized as representing definitive evidence of natural selection in humans. Variation existed in the form of a polymorphism, and a deleterious allele in the heterozygote state conferred a fitness advantage to individuals due to certain environmental conditions (living in malarial areas).

The Mystery Disease

Pre-Laboratory Activities

- Dr. Allison suggested that the frequency of the mystery allele was (and still is) much lower in the African American population in the U.S. due to the absence of malaria in the United States. Since the deleterious allele was not conferring any selective advantage, it was being eliminated from the population. Today, there are regions in Africa where the frequency of the mystery disease allele ranges from 20% - 40%. In the United States, however, the allele frequency remains at about 7-8%.

Allow time for students to develop new explanations concerning if and how the data on malaria shed any light on why there are unusually high frequencies of the mystery disease allele in some regions of Uganda (10-15 minutes). Students should discover three things:

- 1) There is a positive correlation between the incidence of malaria and the frequency of carriers of the mystery disease;
- 2) Children who are carriers of the mystery disease seem to contract malaria less frequently; and
- 3) If children had the mystery disease allele and contracted malaria, their parasite loads were much lower than children who did not have the mystery disease allele.

From these three pieces of information, students should be able to come up with the explanation that the heterozygotes are somehow “protected” from malaria, which results in a higher fitness, so the mystery disease allele remains in the population at a relatively high frequency.

A number of additional **nature of science** concepts can be incorporated into this activity:

- 1) At the end of the activity, ask the students, “What do you think about the fact that you are basing your explanations for the high frequencies of the allele on data collected by observational methods rather than from a controlled experiment? Do you think experiments are necessary for knowledge to develop in science?” This question relates to the misunderstanding some students (and adults!) have that “good” science is based on experiments. Scientists do not have to conduct experiments in order to do “good” science. In fact, some sciences are mainly observational, such as astronomy.
- 2) This activity is very good for explaining to students that science is tentative; it is normal in science for hypotheses to be developed, to gain prominence, and then to be changed or abandoned in light of new evidence.
- 3) Also, mention that there were several competing theories as to why the “mystery” allele persisted in certain parts of Africa, yet all of the scientists had access to them same data. Why do you think this occurred? Again, this relates back to the fact that science is subjective.

Students can be challenged to come up with an explanation for why there is an increased resistance to malaria in those with the mystery disease allele. Why did the carriers for the disease (the heterozygotes in Allison’s research) exhibit lower parasite densities? What mechanism could be at work? Students have learned that the blood of heterozygotes behaves normally – at least, according to the earlier blood tests that were conducted. So, why then are heterozygotes protected from malaria?

The Mystery Disease

Pre-Laboratory Activities

Begin with having the students construct a chart that summarizes their knowledge thus far pertaining to people living in Uganda (10-15 minutes):

Genotype	+/+	+/-	-/-
Red Blood Cell Shape?	Doughnut	Usually Doughnut or Mixture	Crescent
Advantage?	None	Resistant to Malaria	Resistant to Malaria
Disadvantage?	Susceptible to Malaria	None	Anemia/Fever/Disease

A review of the structure/function of red blood cells and proteins/hemoglobin should follow (10-15 minutes). At a minimum, students should understand that the function of red blood cells is to carry oxygen using hemoglobin. In addition, hemoglobin has a high affinity for oxygen when oxygen levels are high (such as in the capillaries of the lungs). However, when oxygen levels are low, hemoglobin has a low affinity for oxygen and is more likely to give up its oxygen molecules. A low oxygen environment exists in the capillaries of the body. Thus, when red blood cells pass through the capillaries in the body, the hemoglobin is willing to “give up” its bound oxygen, which diffuses into the blood plasma and then into the cells surrounding the capillaries due to the partial pressure of oxygen being greater in the capillaries than in the surrounding cells.

Pose the following questions to the students to help transition to the laboratory portion of the activity:

1. Both the earlier *in-vitro* blood test and the later *in-vivo* blood test that you learned about the presence of structural changes in the red blood cells under low oxygen environments. What could be affected within the red blood cells to cause these structural changes? [Students will hopefully conclude that there is some defect in the hemoglobin such that under low oxygen conditions a structural change occurs.]
2. Based on your answer to the question above, what are possible differences between the blood of a normal person, a carrier, and the mystery patient? [The blood of a normal patient has normal hemoglobin. The blood of a carrier has a mixture of normal hemoglobin and defective hemoglobin. The blood of the mystery patient has the defective hemoglobin.]

Activity H: Differential Diagnosis (optional; 10 – 15 minutes)

In this optional activity, students are given the description and symptoms of eight different diseases and must perform a differential diagnosis in an attempt to identify the mystery disease. Students can use the included dry erase markers to underline any information on the cards that they think might be pertinent.

Use this activity if students are still not sure what the disease is after having completed the previous activities. It is also a good lead-in for the gel electrophoresis section of the lab.

Students tend to choose sickle cell anemia as the mystery disease based on this activity; however, some students think it could be either sickle cell anemia or thalassemia based on the symptoms – and they are correct! (Please see page 19 for details on how to differentiate between thalassemia and sickle cell anemia).

The Mystery Disease

Laboratory Explanation

The purpose of the laboratory activity is to apply the concepts developed in the pre-lab to a clinical test for sickle cell anemia using protein gel electrophoresis.

The Objectives of the Laboratory Activity are:

- ♦ Use gel electrophoresis to distinguish normal hemoglobin from sickle cell hemoglobin
- ♦ Interpret gel electrophoresis results
- ♦ Demonstrate the concept and process of gel electrophoresis

Before proceeding with the laboratory investigation, it is necessary to make a logical connection to the concepts developed in the pre-laboratory activities. In doing so, the laboratory activity becomes a tool in the continuum of an ongoing problem rather than an isolated end in itself.

If you did Activity G, then acquaint students with protein gel electrophoresis as a way to test for structural differences in normal hemoglobin, people who are heterozygous for the “mystery disease”, and people who have the full-blown version of the disease. Dr. Linus Pauling, a famous scientist, was the first to run gel electrophoresis on hemoglobin samples in 1949. His results showed that a person with the heterozygous condition had two types of hemoglobin – one that looked like normal hemoglobin and another that looked like that from a person with the full-blown version of the disease. From these results, Dr. Pauling concluded that low oxygen conditions caused the defective version of the hemoglobin to sickle. If Activity G was not done, then doing Activity H (Differential Diagnosis) would be a good way to transition into the laboratory portion of the activity as students usually narrow the “mystery disease” down to sickle cell anemia and thalassemia.

Developing the Concept for the Laboratory Activity

With the understanding of the “mystery disease” generated by the pre-lab, ask students to consider ways to test for the disease. A common response is to examine the blood and look for signs of anemia or sickled cells. Anemia, however, is not unique to sickle cell anemia nor are the blood cells necessarily sickled unless the patient is in crisis. Furthermore, thalassemic blood samples frequently look very similar to sickle cell blood samples (thalassemia is a hemoglobin disorder associated with the defective synthesis of hemoglobin). Because hemoglobin is the molecule affected by the disease, the conclusion is to observe the diseased or affected hemoglobin for characteristics that would distinguish it from normal hemoglobin.

Developing the Concept for Gel Electrophoresis

The next goal is to help the students realize the conceptual basis of the test that will help distinguish normal hemoglobin from affected hemoglobin (sickle cell hemoglobin). Raise the question by holding up a tube containing a sample of “hemoglobin” and ask whether they can identify it as normal or abnormal (use red food coloring and water to create a light rust color which simulates the color of both normal and sickle cell hemoglobin; a sample is included in your kit). The students realize that they first need to see what a normal hemoglobin sample looks like in order to identify whether the unknown is normal. Place control samples of “normal hemoglobin” and “abnormal hemoglobin” next to the unknown sample. Again ask whether they can identify which sample is normal and which is affected by visually comparing the three samples of “hemoglobin”. The samples look exactly alike in the tubes. Therefore, a tool is needed to distinguish between hemoglobin samples that look identical but have different properties. The tool, gel electrophoresis, will be used in the laboratory activity.

The Mystery Disease

Laboratory Explanation

Protein gel electrophoresis can be used because normal hemoglobin protein has a net charge of -2 and sickle hemoglobin protein has a net charge of -1 , and the samples will migrate differently in a gel because of their differences in charge.

Electrophoresis Role-Play

A role-play may be used to demonstrate the theory behind electrophoresis. Have two groups of three students come to the front of the room. Each group represents a hemoglobin protein, and each person represents an amino acid. Note that both molecules have the same number of amino acids and are, therefore, the same size. Give each student a card with a number representing a charge of -1 or 0 . To one group assign two -1 charges and one 0 charge. To the other group give two people 0 charges and one person a -1 charge. Consequently, one group has a net charge of -2 and the other group has a net charge of -1 . Point out that the difference in overall charge between the two molecules cannot actually be seen with the naked eye. However, the charge difference does make the hemoglobin react differently in an electric field. Illustrate this concept by telling the class to imagine the classroom as an electrical field with the positive pole at the back of the room and the negative pole at the front of the room. In an electrical field, the negatively charged hemoglobin molecules migrate toward the positive pole. The group with a net charge of -2 will move more quickly because it has a greater negative charge drawing it toward the positive pole. Pretend to turn on the electricity and have the two groups of students migrate as the molecules would. The groups can be distinguished by their different rates of migration with respect to their net negative charge. To check student understanding, have the students predict and demonstrate the migration if the molecules both had a charge of -2 .

The Laboratory Investigation: Protein Electrophoresis

Students will work in 10 groups. Each group receives three samples of “hemoglobin”: “**A**”=Normal hemoglobin control, “**S**”=Sickle cell hemoglobin control, “**P**”=Patient hemoglobin sample. The patient samples may represent normal hemoglobin, sickle cell hemoglobin, or both in the case of a carrier. The samples of “hemoglobin” are put into an electrical field and the rates of migration compared. The negatively charged samples, either -1 or -2 , will be attracted to the opposite charge and migrate towards the positive electrode in the gel box. The “hemoglobin” samples are really made up of dyes which will migrate through the gels as actual normal and sickle hemoglobin would. The agarose gels and electrophoresis buffer will be prepared in advance, but explain to the students how gels are made. Also, describe how the wells of the gels are made. It is recommended that the students load the gels dry and then add the running buffer. The gels will run at 200 volts for 15 minutes. As the gels run, encourage the students to look through the lid or the side of the electrophoresis box to see their samples start to migrate.

Interpretation of Results

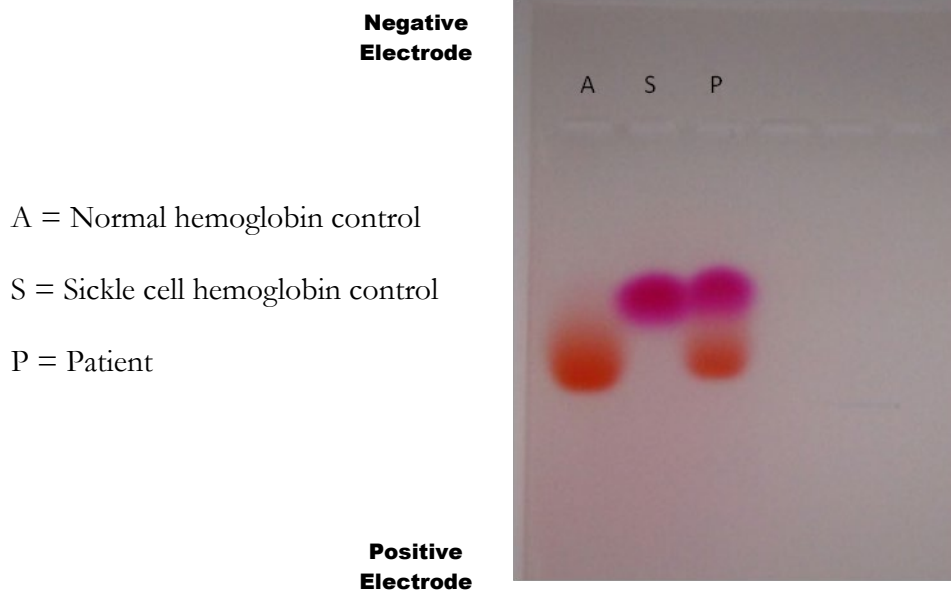
The Normal hemoglobin control will have an orangish-red band that appears lower in the gel because it runs faster with a -2 net charge. The Sickle hemoglobin control will have a pinkish-red band that appears higher in the gel because it does not run as fast with only a -1 net charge. Patient results will vary. Some patient samples will display two bands (one orangish-red and one pinkish-red representative of a carrier), others will be positive for sickle cell anemia (with only a pinkish-red band), while some will be negative for sickle cell anemia (with only an orangish-red band). Students may notice that the bands are different colors. This is because we use a dye and not real hemoglobin for this lab. You can handle this with your students in two ways: either inform them that we are using dyes and not real hemoglobin and that real hemoglobin does not differ in color between normal and sickle, or you could tell the students that the color is not a reliable indicator.

The Mystery Disease

Laboratory Explanation

Either way, make sure the students are only looking at distance migrated, rather than color, when interpreting results. Students should draw their results in the diagram on the lab protocol sheet.

Picture of Hemoglobin Gel Electrophoresis Results:



To facilitate discussion, choose a representative gel of each outcome and put the gels on an Elmo if available. Highlight the bands projected on the board with a marker. Some sample questions for discussion include:

- What can be inferred from the results of the test?
- How can the presence of two bands in some patient samples be explained?

Some student groups may have discovered that their patient was “normal”. So, how would they explain the fact that this patient exhibited the same symptoms as those of Dr. Herrick’s mystery patient who, it turned out, had sickle cell anemia? Have they learned about anything previously that might explain this?

If the students did the optional Differential Diagnosis activity (Activity H), they should have learned that people with thalassemia have many of the same symptoms as those with sickle cell anemia. Also, thalassemic blood samples frequently look very similar to sickle cell blood samples. Thalassemia is a hemoglobin disorder associated with the defective synthesis of hemoglobin. It results in reduced, or sometimes absent, *quantities* of hemoglobin. The hemoglobin of people with alpha-thalassemia will look “normal” with the test that the students ran in the lab. Other tests would have to be run that test for: the *quantities* of hemoglobins A, F, H, and A₂; the concentration of red blood cells within a blood sample; and the overall amount of hemoglobin in a blood sample.

The Mystery Disease

Post-Laboratory Explanation

Post-Laboratory Explanation (10 – 15 minutes)

After all of the groups have completed the activities and the laboratory portion, ask students to develop an explanation for the mechanism of the disease. Next, ask each group to present their explanations to the entire class. Encourage students to be creative in their presentations by giving them the option to present verbally, in writing, with diagrams or concept maps, or by using role-play. Students often generate many ideas and interesting topics for discussion. Encourage students to debate their ideas and consider them in light of the observations they made. Challenge and elaborate on students' ideas to lead them to discover the following points:

- The blood cells are irregularly shaped (seen in photo)
- The irregular shape of the red blood cells interferes with their ability to flow through the blood pathways (inferred by the activity with the flasks and red blood cells made of clay)
- The condition is inherited (as seen with the pedigrees)

Refer to the activities to assist the students' discovery of the above points. Activity B indicates anemia and irregularly-shaped red blood cells as seen in the photo. The blockage created in blood vessels by the sickled cells is illustrated by the normal and sickled red blood cells in the flasks in Activity C. From Activities D and F, the family history suggests the possibility that the condition is inherited.

Several **nature of science** concepts can be incorporated into the discussion at the end of the lab:

- 1) Ask students if they think that scientists now know all there is to know about sickle cell anemia. This question relates to the nature of science concept that science is tentative yet reliable. It took researchers until 2011 to figure out why people with sickle cell trait are less susceptible to malaria. Why? Because they didn't have the technology necessary back in the 1940s and 1950s – they needed an electron microscope! (Please see November 2011 *Nature* article by Meredith Wadman in relation to this discovery at: <http://www.nature.com/news/sickle-cell-mystery-solved-1.9342>)

- 2) If you presented this laboratory activity in the manner suggested above (from a historical perspective), ask the class, "How does scientific knowledge develop?" Students, hopefully, have picked up on the fact that scientists learn about things a little at a time; science involves trial and error, and hypotheses and theories can change over time as new evidence is found.

The Mystery Disease

Micropipette Instructions

Micropipettes

Micropipettes are precision instruments designed to measure and transfer small volumes of liquid. They are expensive and must be used with care. Their accuracy depends upon their proper use. Different brands of micropipettes vary in the volume range they will measure, the type of tips they fit, and the type of device used to set the volume. Be sure that everyone understands how to operate the micropipettes correctly.

Golden Rules of Pipetting

- 1. Be aware of the upper and lower range of the pipette. Going above or below the range will damage the micropipette.*
- 2. Always use the micropipette with a micropipette tip. Without a tip on the end, liquid can get into the opening of the pipette and damage the mechanism inside.*
- 3. Always hold the micropipette straight up to prevent liquid from getting into the micropipette.*
- 4. Use new pipette tips between different samples to prevent contamination.*

Basic Directions for Micropipette Use

Setting the Volume

All micropipettes have a volume control dial. Determine whether the volume window on your pipette shows tenths of microliters (0.1 μl) or whole microliters in the smallest place, so that you can read the scale correctly (it varies with different brand micropipettes).

Drawing Up and Expelling Liquid

Micropipettes have 2 stops as you press down on the plunger to expel liquid. The first stop corresponds to the volume set in the window. The second stop gives a little puff of air to blow out any remaining liquid upon delivery. To draw liquid into the pipette tip, press down on the plunger only to the first stop. If you go to the second stop you will draw too much liquid into the tip. The most common pipetting error is to go past the first stop to the second stop for drawing liquid into the tip (which gives an inaccurate volume). When you are letting the liquid out of the tip, then you go to the second stop. It is worthwhile to check each student for correct technique before beginning laboratory procedures that require the use of the pipettes.

The Mystery Disease

Micropipette Instructions

Using the Micropipette:

1. Select the pipette that includes the volume range you will need.
2. Adjust the pipette to the desired volume by turning the dial. Do not turn beyond the volume range for the pipette.
3. Press a new tip onto the pipette firmly (gently tap the pipette into a tip while in the box). Get a tip without touching it with your hands; this is to prevent contamination of the samples.
4. To draw liquid into the micropipette tip:
 - a) Press down the plunger to the first stop to measure the desired volume and hold in that position.
 - b) Holding the pipette vertically, immerse the tip 1-3 mm into the liquid to be transferred.
 - c) Draw the fluid into the tip by slowly releasing the plunger. Wait 1-2 seconds to be sure that the full volume of sample is drawn into the tip. If you see air bubbles, there is a problem with your volume and you will need to repeat this step to get the correct volume (either your tip wasn't immersed far enough down into the liquid or you perhaps raised your arm while releasing the plunger).
5. To dispense the liquid:
 - a) Place the tip into the container where the liquid is to be released.
 - b) Slowly press down the plunger to the second stop to blow out all of the liquid in the tip. Be careful not to suck liquid back into the tip by releasing the plunger while the tip is in the liquid you just dispensed.
 - c) Eject the tip when done into a waste container by pressing the separate ejector button found on the top or side of the micropipette (depends on the brand of micropipette).

The Mystery Disease

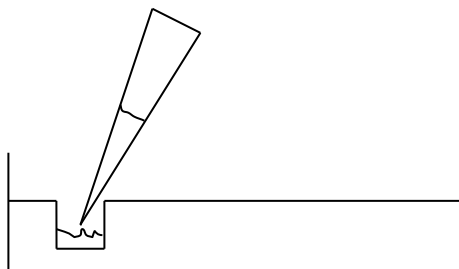
Practice Gel Loading Exercise

Loading gels, or filling the wells of a gel, can be a challenging task, especially if one has never done it before. This is an opportunity to practice before you are asked to load the actual samples involved in the laboratory activity. Take your time, figure out how you feel most comfortable doing this (example: some people like to rest their elbow on the counter while loading), and practice filling a few different wells of the practice gels. Remember, this is for practice, so don't get frustrated if liquid spills out of a well or if you accidentally tear the edge of the agarose gel – just try it again.

An important thing to note about gels: the wells appear as holes but they really aren't. They are more like indentations that do not go through completely to the bottom of the petri dish. This is why it is so important not to poke the micropipette tip through the bottom of the well or the liquid will seep into the bottom of the dish and not stay in the well.

Loading the Practice Gels:

1. Become familiar with the feel of pressing down the plunger until it stops.
2. Then set the micropipette to **10 μ l**.
3. Make sure you gently tap a tip onto the end of the micropipette.
4. Remove the lid of the practice agarose gel and make sure you can clearly see the wells.
5. To suck up the practice loading dye into the tip, press down on the plunger until it stops, then place your tip into the liquid dye and slowly lift up your thumb. (Be careful not to raise your hand while lifting your thumb or you'll get air bubbles and the volume will be incorrect.)
6. Select a well to pipette the dye into.
7. Lower the tip filled with the dye into a well to be filled. Be careful not to poke through the bottom of the well or rip between the wells or the liquid will not stay in the individual well you chose.



8. To release the dye from the tip, press down on the plunger until it stops. Next, lift up the micropipette so the tip is no longer in the well (or you may accidentally suck the liquid back into the tip).
9. Look to see if all of the dye went into the well.
10. Repeat this at least two or three times until you feel comfortable loading samples into a well. Each person in the group needs to practice loading wells in the practice gel. You do not need to change tips since you will be using the same liquid between group members.

The Mystery Disease

Teacher Laboratory Preparation

A PowerPoint presentation to support this activity is available on the Maryland Loaner Lab page (<https://www.towson.edu/fcsm/centers/stem/loanerlab/>).

Maryland Loaner Lab will supply reagents, equipment, and instruction for the laboratory activity for up to 10 groups. **Teachers must supply distilled water used for making gels and buffer. Teachers must supply the students with the following handouts:** Pre-Laboratory Activities A-G; Laboratory Protocol; and Student Worksheet.

Prepare Student Stations (10):

- Foam microcentrifuge tube rack
- One tube each: “A”, “S”, and “P”
(Use the microcentrifuge to spin down all samples for 2 seconds.)
- Box of micropipette tips (1 box to be shared between 2 student groups)
- One practice gel
- One practice loading dye tube
- One disposable cup (waste container for tips) **(Provided by the teacher.)**

Activity:	Time needed:
Preparing Gels & Student Stations	30 minutes
Pre-Lab Activities	70-95 minutes
Practice Gel Loading Exercise	15 minutes
Laboratory Activity	30 minutes
Post-Lab Activity	10-15 minutes

Shared Equipment for Multiple Groups:

- One agarose gel for every 2 groups (each group will use 3 wells)
- One gel electrophoresis chamber (gel box) for all 10 groups
- One power supply for the gel box

Electrophoresis: Gel Preparation and Directions for Running Gels

Step 1 – Prepare 1X TAE Buffer (for making agarose gels and for use as a running buffer)

Buffer (not water) must be used to make and run the gels. The buffer supplies the necessary ions to conduct electricity. The buffer received in the kit is 10X Tris-Acetate-EDTA (TAE) in a 150 ml bottle (150 ml total), and needs to be diluted with distilled water (dH₂O) to make a 1X concentrated solution.

Add the entire 150 ml of 10X TAE buffer (entire bottle) to 1350 ml of distilled water in the 2-liter container provided and mix well. From this now diluted 1X TAE buffer, 120 ml will be used to make the agarose gels and 1000 ml will be used as the electrophoresis running buffer.

Step 2 – Prepare a set of six 0.7 % agarose gels (5 gels for the activity with 1 extra gel).

Agarose gels and running buffer may be made the night before use. This prep will make 6 small gels. Each gel will have 8 wells and will accommodate 2 groups of students with 3 samples each. Before making the agarose solution, have casting trays prepared and ready to be used (see Step 3).

The Mystery Disease

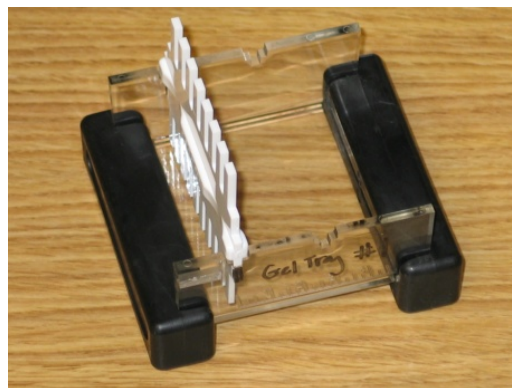
Teacher Laboratory Preparation

Pour the entire contents of the microcentrifuge tube containing 0.84 g of agarose powder found in the powder bag into the orange-capped glass bottle. Then, add 120 ml of the diluted 1X TAE buffer from Step 1 (use the graduated cylinder). Add the buffer to the glass bottle, and mix well with the agarose powder by swirling the bottle.

1. Dissolve the agarose in a microwave or on a hot plate. **The orange bottle cap must be removed before heating.** The power of the microwave may vary, but to prepare 120 ml of agarose it generally takes 1.5-2 minutes on high power. For best results place the bottle in the microwave for one minute, stir and heat for 30 more seconds, stir and heat another 30 seconds only if needed. **Do not over heat** as the liquid will boil out of the bottle and spill. It is best to microwave in small time intervals and mix, then continue heating. The agarose must be completely dissolved in solution and well mixed. No particulate matter should be visible.
2. Cool the agarose solution to about 60°C by placing the melted agarose in a 60°C water bath or by allowing it to sit at room temperature for several minutes. **Swirl occasionally** while it is cooling to avoid rapid cooling of the agarose in the bottom of the bottle so that the agarose does not start to solidify (or reheating will be necessary). The bottle of melted agarose solution is ready to be used when it is warm to the hand but not too hot to handle (if it's too hot it can warp the comb and gel tray).

Step 3 – Casting Agarose Gels

1. Place the rubber dams onto the ends of each gel tray (it is easiest to lay the rubber dam on a table and, holding the gel tray, carefully press it into one corner and then use your weight to “roll” the gel tray into the second corner and repeat with the other rubber dam). **Use caution to prevent breaking the gel tray.**
2. Place the gel trays with rubber dams onto a flat surface.
3. Position the comb teeth down over the black mark. **Use the large teeth only, as it will create 8 wells of the needed size.**
4. Swirl the mixture and slowly (to avoid air bubbles) pour 20 ml of cooled agarose solution into each of the 6 casting trays using a graduated cylinder (use a pipette tip to pop any air bubbles).
5. After the gel has hardened (about 30 minutes), gently remove the comb. It is important that the gels have completely solidified before the comb is removed.
6. Being very careful so that the gel does not slide off the gel tray, remove the two rubber dams from each end of the gel tray.
7. The gels may be stored by placing them in a zip-lock bag or other plastic container. Refrigeration is best, but not required.



Step 4 – Prepare Electrophoresis Running Buffer

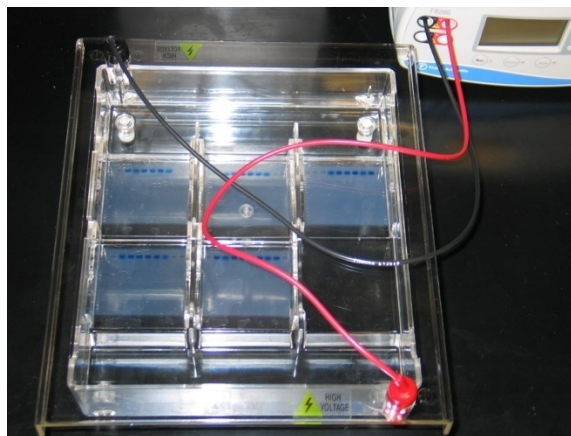
Measure out 1000 ml of 1X TAE buffer from Step 1. This now is the electrophoresis running buffer that will be used to run the gels. The gel box requires approximately 1000 ml of running buffer. The buffer may be stored at room temperature or in a refrigerator.

The Mystery Disease

Teacher Laboratory Preparation

Step 5 – Electrophoresis of the Samples (following student Laboratory Protocol)

1. The electrophoresis gel box holds all six gel trays. The gel trays are labeled “1-6” with one being extra. Assign up to two student groups on one gel tray and assign three wells per group. Each gel has 8 wells, so assign wells #2-4 and wells #6-8 to the two student groups using each gel.
2. Next, the gels will be loaded dry at the students’ tables. Students will load 20 μl of the hemoglobin samples to their assigned wells. Finally, be very careful picking up the gel trays and adding them to the gel box (notice there is a notch at the top of the gel tray that fits or “locks” into place in the gel box). Be sure to **place the gel trays in the gel box so the ends containing the wells are closest to the black electrode** or the samples will run backwards. This gel box holds two rows of gel trays so both rows must be oriented the same way in the gel box (see picture).
3. Next, slowly pour 1000 ml of the **1X TAE running buffer** into the bottom chamber of the gel box (nearest the red electrode). **Do not pour the buffer directly onto the gel** or the samples may come out of the wells. The gels in the trays need to be completely submerged to run, but the top of the trays (sides) will be exposed out of the buffer while running.
4. Once gels have been placed in the gel box and the running buffer added, be careful not to disturb the electrophoresis apparatus.
5. Place the cover on the gel box matching black and red electrodes.
6. Connect the gel box lid to the power supply, again matching black and red electrodes to the colors marked on the ports of the power supply.
7. Follow the printed directions found on the top of the power supply to start the run. The voltage selector on the power supply should be set to 200 V, and your timer should be set for 15 minutes.
8. To confirm proper operation of the power supply, look for bubbles rising from the electrodes and that the samples are moving in the proper direction (“running towards the red”).
9. When the gels are done, turn off the power supply and disconnect the lid of the gel box from the power supply.
10. Remove the gel trays from the box.
11. The hemoglobin bands are best visualized when viewed against a white background or even better on a light box.
12. When done, the running buffer may be poured down a sink drain. Used gels can be disposed of in the trash.
13. After use, the gel box and trays should be rinsed with tap water and allowed to air dry.

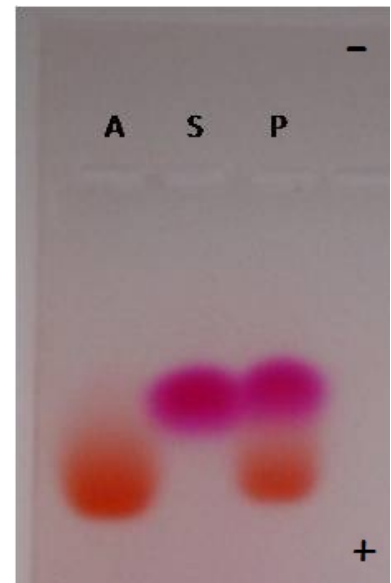


The Mystery Disease

Teacher Laboratory Preparation

Step 6 – Interpretation of Results

The Normal hemoglobin control will have an orangish-red band that appears lower in the gel because it runs faster with a -2 net charge. The Sickle hemoglobin control will have a pinkish-red band that appears higher in the gel because it does not run as fast with only a -1 net charge. Patient results will vary. Some patient samples will display two bands (one orangish-red and one pinkish-red representative of a carrier of the sickle cell trait), others will be positive for sickle cell anemia (with only a pinkish-red band), while some will be negative for sickle cell anemia (with only an orangish-red band). Students may notice that the bands are different colors. This is because we use a dye, and not real hemoglobin, for this lab. You can handle this with your students in two ways: either inform them that we are using dyes and not real hemoglobin and that real hemoglobin does not differ in color between normal and sickle, or you could tell the students that the color is not a reliable indicator. Either way, make sure the students are only looking at distance migrated, rather than color, when interpreting results. Students should draw their results in the diagram on the lab protocol sheet.



Example of Hemoglobin Gel Electrophoresis Results – in this example, the patient (P) is a carrier for the sickle cell trait.

The Mystery Disease

Activity A Answer Key

1. What symptoms do you find concerning?

Answers can include: becoming so tired after swimming that he could hardly move; shortness of breath; pain in joints and muscles; unusual weakness; requiring bed rest for several weeks; symptoms occurring repeatedly; frequent fevers and infections; fatigue and soreness in joints; white of eyes had yellow tint [jaundice]; and pain in left abdominal area [due to damage to spleen].

2. Are the patient's various symptoms related to one another? If so, how? If not, explain why.

At this point, students may disagree as to whether the symptoms are all related to one another. One way in which students might say the symptoms are related is if they think that the symptoms are related to a problem with the blood or cardiovascular system. Shortness of breath and fatigue can point to a cardiovascular problem. Pain/soreness in joints and muscles can occur if the blood is clotting in the muscles and joints. Frequent fevers and infections can occur if a person's white blood cell count is low (as occurs in those with sickle cell anemia). Jaundice can occur if there is excess bilirubin (a yellow chemical) in the blood due to the excessive breakdown of red blood cells, which are processed by the liver. However, if there are too many red blood cells dying (as in the case of sickle cell anemia), bilirubin builds up in the body and causes a person's skin and the whites of their eyes to turn yellow. Pain in the left abdominal area is most likely due to a splenic sequestration, which occurs when sickled red blood cells block the blood vessels leading out of the spleen, causing blood to stay in the spleen instead of flowing out of it. When this occurs, the blood count (hemoglobin and hematocrit) falls and the spleen enlarges, which can sometimes be painful.

3. What would you do to further study this mystery disease?

Hopefully, by this time, you have been able to lead the students to the understanding that the patient's symptoms are consistent with a blood or cardiovascular problem. Thus, students should suggest looking at a blood smear or something related.

The Mystery Disease

Activities B and C Answer Keys

Activity B Answer Key

1. What differences do you see between the patient's blood and the normal blood?

There are fewer red blood cells and more white blood cells (in purple) in the mystery patient's blood. Some of the mystery patient's red blood cells are oddly-shaped and some are clumped together.

2. How might these changes affect a person's circulatory "health"?

Students might suggest that the clumped and oddly-shaped red blood cells might cause problems with blood flow – the cells might get stuck in the blood vessels. Lack of enough red blood cells would mean that the patient is not getting enough oxygen to his cells. The severity of sickle cell anemia increases with increased white blood cell (leukocyte) count. These impaired white blood cells adhere to the walls of blood vessels, clump with other blood cells to increase blockage of blood vessels, resulting in tissue damage and inflammation. The white blood cells of a person with sickle cell anemia are also impaired in their ability to kill microbes, resulting in increased frequency of fevers and infections. The teacher could then ask students how this might relate to the patient's symptoms. (Blood clots can lead to pain in the muscles and joints. Lack of oxygen can lead to extreme fatigue, weakness, and shortness of breath. An increase in leukocytes could indicate more frequent fevers and infections.)

Activity C Answer Key

1. What do each of the different-sized flasks represent?

There is some leeway regarding what each flask represents. The largest flask represents arteries, the middle flask can represent veins or arterioles, and the smallest flask represents capillaries.

2. Explain how you will go about exploring the relationship between red blood cell morphology and blood flow in arteries, veins, and capillaries.

This activity is fairly open-ended. The idea is for the students to determine what's going on in the blood vessels of the mystery patient versus those of a normal person. Students can vary the ratios of normal to sickled red blood cells and transfer them from one flask to another to see what happens.

3. After conducting the activity, explain what you have discovered.

Students should discover that the sickled red blood cells get stuck where the neck begins in the smallest flask (the capillaries).

The Mystery Disease

Activities D and E Answer Keys

Activity D Answer Key

1. Is this disease inherited? *Yes*
 - a. If so, what type of inheritance accounts for the pattern seen in the pedigree?
What is your evidence?
The disease seems to be autosomal dominant because many members of the mystery patient's family tested positive using Emmel's test. If students were to do a Punnett Square, they would find the results consistent with that of an autosomal dominant inheritance pattern.
 - b. If you do not think that the disease is inherited, what is your evidence?
Student responses can vary.

Activity E Answer Key

1. Based on the information you just read, was anything problematic regarding this type of test? Explain.
Emmel's test was an in-vitro test. It did not mimic the conditions within the body. The microscope slide environment did not have the correct temperature, pH, pressure, amount of oxygen, etc.
2. How did Emmel's test depart from our current understanding of blood physiology?
Even outside of the body, blood continues to metabolize oxygen. However, after 48 hours, there would be no more oxygen to metabolize.
3. Can you explain why some of the mystery patient's family members' blood changed shape under Emmel's test conditions?
At this point, students should understand that there seems to be a link between lower, or a complete lack of, oxygen and the shape change in some of the family members' blood. [A more detailed explanation for the teacher...A person who is heterozygous for sickle cell anemia, or who is said to have the "sickle cell trait", can also have sickling crises if the amount of oxygen in their blood is too low, which would occur if their blood was left on a microscope slide enclosed by a wax ring for two days.]

The Mystery Disease

Activities F and G Answer Keys

Activity F Answer Key

1. Why is an in-vivo test preferable?

An in-vivo test is preferable because it closely mimics the conditions in the body, thus giving more accurate results. It also allows one to distinguish between full sufferers of the disease and those who only exhibit mild symptoms.

2. Given this pedigree, do you believe that genetics still plays a role in the transmission of the disease? If so, how?

Students will usually either say that the disease is still inherited, but it now looks to be homozygous recessive or they will state that they no longer think that the disease is inherited because there are now so few family members that have it. If some students no longer believe that the disease is inherited, the teacher could ask the students who do still believe that it is inherited to try to persuade their classmates that they are correct and vice versa. Evidence to support a conclusion that the disease is homozygous recessive include: one would expect to find much fewer people with the condition if it is recessive; the genotypes of the various individuals on the pedigree can be worked out using a recessive model (you could have the students try this themselves); and one would expect to find that about 25% of the offspring of two heterozygote individuals for the disease would have the disease themselves.

3. How does the technique used to determine this pedigree differ from the technique used to determine the previous pedigree?

The newer technique uses a procedure that more closely mimics the conditions that a blood cell would be exposed to while in the body.

4. Was the hypothesis that you developed based on the 1923 pedigree data “wrong”? Explain.

Students should understand that the hypotheses that they came up with based on the results of Emmel’s test were not “wrong”; they were simply the best explanation that they could come up with given the current knowledge and technology. Science progresses with the accumulation of more knowledge and more advanced technology.

Activity G Answer Key

Before you begin, answer the following question:

1. As scientists, you all have access to the same data for this problem. Do you think that you will all come up with the same explanation for the unusually high frequencies of this allele? Why or why not?

Students should understand that they will probably not all come up with the same explanation for why there is an unusually high frequency of the allele for the mystery disease. Just like scientists, students all have different backgrounds (cultural, socioeconomic, ethnic, etc.), prior knowledge, and past experiences, all of which influence how they “see” the data.

The Mystery Disease

Activity G Answer Key

2. Do you think these new data shed any light on why there are high versus low frequencies of the mystery disease in various areas of Uganda? Explain.

The results indicate that fewer children who were carriers for the disease had the malaria parasites, and in those who did have the parasites, the density of the parasites in the blood was lower. Thus, it seems that being a carrier for the mystery disease somehow confers an advantage to that person by protecting them from malaria.

3. Do you find it troubling that you are basing your explanations for the seemingly high frequencies of the mystery disease on data collected using observational methods rather than controlled experiments? Why or why not?

Students should understand that it is not necessary to conduct controlled experiments in order to do “good” science. In fact, there are fields of science in which it is nearly impossible to conduct controlled experiments, such as astronomy. Drawing inferences from observations is perfectly legitimate science.

Data Analysis and Conclusions:

To help you with the following questions, fill in the following chart based on what you’ve learned about the mystery disease:

Genotype	+/+	+/-	-/-
Red Blood Cell Shape?	Doughnut	Usually Doughnut	Crescent
Advantage?	None	Resistant to Malaria	Resistant to Malaria
Disadvantage?	Susceptible to Malaria	None	Anemia/Fever/Disease

4. Both the earlier in-vitro blood test and the later in-vivo blood test that you learned about measured the presence of structural changes in the red blood cells under low oxygen environments. What could be affected within the red blood cells to cause these structural changes?

This question requires an understanding of hemoglobin and its function. If students understand that the function of hemoglobin is to carry oxygen, they may then conclude that there is some kind of defect in the hemoglobin that causes the hemoglobin to change shape under low oxygen conditions.

5. Based on your answer to question #4, what are possible differences between the blood of a normal person, a carrier, and the mystery patient?

The blood of a normal person has normal hemoglobin. The blood of a carrier for the mystery disease has a mixture of normal and defective hemoglobin. The blood of a person with the full-blown mystery disease just has the defective hemoglobin.

The Mystery Disease

Activity G and Laboratory Protocol Answer Keys

6. Why do you suppose there is an increased resistance to malaria in those who are heterozygous for the mystery disease allele?

This question may be a little difficult for the students, but they can at least think about it and make some guesses. The answer is that the parasite cannot reproduce in a red blood cell containing the mutated sickle cell hemoglobin.

7. Do you think experiments are necessary for knowledge to develop in science?

No, they're not. Inferences drawn from observations are perfectly legitimate science and help science progress. A good example of this is astronomy. Much of the knowledge gained in this field has come from observational data.

8. Has your hypothesis to explain the frequencies changed? If so, what caused it to change? If not, why not?

Students will probably answer that their hypothesis has changed due to the accumulation of more information regarding the disease, such as the data regarding the connection between malaria and the mystery disease, the results of Anthony C. Allison's research, etc.

Laboratory Protocol Answer Key

4. Observe the banding patterns on your gel. Do you see a difference between the normal and sickle cell hemoglobin controls? Compare the patient's hemoglobin to the normal and sickle cell controls; which one is it similar to?

Students should focus on the differences in the distances migrated by each sample, not the color differences. Students should notice that the normal hemoglobin sample travels farther down the gel than the sickle cell hemoglobin sample.

The students' patient samples will vary, so answers will vary; some patient samples will look like the normal hemoglobin control; others will look like the sickle cell hemoglobin control; while yet others will resemble the banding pattern of a carrier for the disease (there will be two bands).

6. What is the function of the agarose gel?

The agarose gel holds the samples and serves as a matrix in which different molecules can be separated. In this lab, different hemoglobin proteins (normal and sickle) are separated by their difference in charge.

7. Why is the gel in electrophoresis buffer?

The gel is in electrophoresis buffer to conduct electricity through the gel.

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

Normal hemoglobin has a charge of -2, while sickle cell hemoglobin has a charge of -1; since both normal and sickle cell hemoglobin are negatively charged, they will both move towards the positive electrode (red) because opposite charges attract.

The Mystery Disease

Laboratory Protocol Answer Key

9. Why must you be careful of when loading the samples into the wells of the gel?

You must be careful not to damage the gel (accidentally poke, rip, or tear the gel) and to make sure all of your sample goes into the well and hasn't spilled out of the well.

10. Even though the hemoglobin in the normal and sickle controls look very similar in the tubes, how do they look different in the gel (talk about migration of bands, not colors)?

The normal hemoglobin will appear lower in the gel because it has traveled farther down the gel due to its greater negative charge of -2 and thus its stronger attraction to the positive electrode than the sickle cell hemoglobin. The sickle cell hemoglobin will appear higher in the gel (closer to the wells) because it hasn't traveled as far down the gel as the normal hemoglobin due to its smaller negative charge of -1.

11. What is your diagnosis for your patient?

(Results will vary among student groups). If the patient sample (P) has sickle cell hemoglobin, then the diagnosis would be sickle cell anemia based on this test result and the patient's symptoms. If the patient sample has both normal and sickle cell hemoglobin, then the diagnosis would be that of a carrier for sickle cell anemia (also known as sickle cell trait). If the patient sample has only normal hemoglobin, then the diagnosis is unknown as only sickle cell anemia can be ruled out. Based on the patient's symptoms, they have some disease state, but cannot be accurately diagnosed by this test, so additional testing will need to be performed. (If students did Activity H – Differential Diagnosis, then ask them, based on what they read about the other potential diseases, which disease it could be? At least one group of students usually guesses that it might be thalassemia, which is correct.)

12. 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.

Students should explain all aspects of gel electrophoresis: how the gels hold the samples, why electrophoresis buffer and electricity is used, what happens to the differently charged hemoglobin molecules, and how normal and sickle hemoglobin controls are used to help determine the patient's hemoglobin results.

The Mystery Disease

A Closer Look at Sickle Cell Anemia Answer Key

1. Transcribe the DNA sequence for five amino acids of a NORMAL hemoglobin gene from the previous page. Write the mRNA sequence below.

CUG ACU CCU GAG GAG

2. Translate the mRNA sequence into its corresponding amino acids. Use the chart of mRNA codons on the next page. Write each amino acid in a box below.

Amino Acid Sequence for NORMAL hemoglobin:

Leu	Thr	Pro	Glu	Glu
<u> 0 </u>	<u> 0 </u>	<u> 0 </u>	<u> -1 </u>	<u> -1 </u>

4. Transcribe the DNA sequence for the same five amino acids of a SICKLE hemoglobin gene from the previous page. Write the mRNA sequence below.

CUG ACU CCU GUG GAG

5. Translate the mRNA sequence into its corresponding amino acids. Use the chart of mRNA codons on the next page. Write each amino acid in the boxes below.

Amino Acid Sequence for SICKLE hemoglobin:

Leu	Thr	Pro	Val	Glu
<u> 0 </u>	<u> 0 </u>	<u> 0 </u>	<u> 0 </u>	<u> -1 </u>

7. Do they differ? If so how?

Normal hemoglobin has a net negative charge of -2, while sickle hemoglobin has a net negative charge of -1 due to the substitution of a neutral valine in place of a negative glutamine.

8. How do you think the differences affect protein function?

Because sickle hemoglobin has a lower net negative charge, it will not be as strongly attracted to the positive electrode when running in the gel, thus it will not move as far down the gel as the normal hemoglobin will.

The Mystery Disease

Extension Activities

Discussion Topics

The investigation can serve as the centerpiece for a variety of topics to be explored further:

Central Dogma

Using the amino acid sequence of the affected and normal hemoglobin, students can identify the mutation from glutamic acid (also known as glutamate) to valine that results in the change in net negative charge of the affected hemoglobin. Working back through the central dogma, they can identify the point mutation in the DNA resulting in the amino acid alteration.

Inheritance

Genotypes can be derived from the phenotypic results expressed on the gel, and the probability of inheriting sickle cell anemia can be predicted given the genotypes of the parents.

Natural Selection

The sickle cell allele is more prevalent in races whose gene pools originated in tropical areas. People of African, Asian, and Hispanic-Caribbean descent have a higher incidence of sickle cell anemia. Selective pressure for the allele results from its ability to decrease the mortality rate of people infected with malaria. Malaria is caused by a parasite in the genus *Plasmodium*, which is transmitted to human hosts by mosquitoes. *Plasmodia* infiltrate red blood cells where they multiply and eventually rupture the cell. Cells with sickle cell hemoglobin are less susceptible to infection by *Plasmodia*. Therefore, carriers of the sickle cell trait (heterozygotes) benefit from the presence of sickle cell hemoglobin while remaining largely asymptomatic (some heterozygous individuals may show mild symptoms) with respect to sickle cell anemia.

Random Mutation/Selective Pressure

Plasmodium did not infect humans 10,000 years ago; it was an avian pathogen. However, a mutation in *Plasmodium* enabled it to jump species and infect humans. Ask students whether they think anyone could have had sickle cell anemia prior to 10,000 years ago. It often leads to a discussion concerning random mutations and the distinction between a Lamarckian and Darwinian perspective on evolution.

Treatments

Ask students how they would treat sickle cell anemia based on their knowledge of the disease. Bone marrow transplants, blood transfusions, and gene therapy are often mentioned. Recently, attention has been given to turning on the fetal hemoglobin gene, which is turned off shortly after birth. Hydroxyurea, which has been used to treat cancer and blood disorders, has been found to stimulate the production of fetal hemoglobin.

The Mystery Disease

Extension Activities

Other Activities

- Use the documents beginning on the next page titled, *A Closer Look at the Cause of Sickle Cell Anemia*. This activity shows students how the sickle cell trait is determined at the genetic level. Have students identify the point mutation in the DNA resulting in the amino acid alteration.
- Sickle cell anemia is an example of one genetic condition for which there is a test but no cure. Have groups of students research other inherited conditions for which there is a test but no cure - for example; cystic fibrosis, Huntington's disease, muscular dystrophy, and fragile x syndrome. Each group can make an informative display about the disease. The display could be in the form of a poster, mobile, booklet, radio broadcast, interview, role-play etc. After each group has made a presentation about the disease, create a role-play in which a genetic counselor presents a scenario, for example:

Both parents are carriers for sickle cell anemia, cystic fibrosis, or any recessive disorder. The couple decides whether or not to try to have children.

One spouse's parent has been diagnosed with Huntington's disease. The couple has two young children. The counselor asks whether they want to be tested.

The role-plays can lead to interesting discussions among the students. The teacher can facilitate by writing down ideas and issues on the board as they come up.

- Howard Hughes Medical Institute (HHMI) BioInteractive has a film about sickle cell anemia and its connection to malaria titled *The Making of the Fittest: Natural Selection in Humans*. The film and film's curriculum guide can be found here:
<http://www.hhmi.org/biointeractive/making-fittest-natural-selection-humans>.

Suggested readings:

- American Society of Hematology: Sickle Cell Disease -
<http://asheducationbook.hematologylibrary.org/content/2004/1/35.full.pdf+html>
- Seminar on Sickle-cell Disease - http://ac.els-cdn.com/S014067361061029X/1-s2.0-S014067361061029X-main.pdf?_tid=29ef7298-58b4-11e6-ad93-00000aacb361&acdnat=1470144330_85ad35a9538022abf51c5412e3772464

The Mystery Disease

A Closer Look at the Cause of Sickle Cell Anemia

Your research has determined that sickle cell hemoglobin differs from normal hemoglobin in the net negative charge of the proteins. This discovery is an important one; it identifies a characteristic that can be used to diagnose sickle cell anemia. However, it does not tell us what causes sickle cell anemia or why the proteins are different. Advances in molecular biology and our understanding of DNA in the past two decades have provided us with more insights into the cause of sickle cell anemia. See if you can use the following data obtained from research in molecular biology to uncover more information about sickle cell anemia.

Document 1: The DNA base sequences of the first seven amino acids for both normal and sickle cell hemoglobin

Document 2: A chart of mRNA codons and their corresponding amino acids

Document 3: The structural formulas for the amino acids and their corresponding charges

Document 1: The DNA Base Sequences of the First Five Amino Acids for Normal and Sickle Cell Hemoglobin

The DNA sequence of bases for the first 5 amino acids in **normal** hemoglobin is:

5' CTGACTCCTGAGGAG 3'
3' GACTGAGGACTCCTC 5'

The DNA sequence of bases for the first 5 amino acids in **sickle cell** hemoglobin is:

5' CTGACTCCTGTGGAG 3'
3' GACTGAGGACACCTC 5'

Here is an example of how to translate DNA sequence into the resulting amino acid:

5'...A T G G C C T G G A C T T C A...3' Sense strand of DNA
3'...T A C C G G A C C T G A A G T...5' Antisense strand of DNA



Transcription of Antisense Strand

5'...A U G G C C U G G A C U U C A...3' mRNA



Translation of mRNA

Met-Ala-Trp-Thr-Ser- **Peptide**

The Mystery Disease

A Closer Look at the Cause of Sickle Cell Anemia

Transcribe and Translate the Segments of the Normal and Sickle Cell Hemoglobin Alleles

1. Transcribe the DNA sequence for the first 5 amino acids of a NORMAL hemoglobin gene from the previous page. Write the mRNA sequence below.

2. Translate the mRNA sequence into its corresponding amino acids. Use the chart of mRNA codons on the next page. Write each amino acid in a box below.

Amino Acid Sequence for NORMAL hemoglobin:

--	--	--	--	--

3. Record the charge of each amino acid on the line provided below each box. Refer to **Document 3: The Structural Formulas for the Amino Acids** for assistance.
4. Transcribe the DNA sequence for the first 5 amino acids of a SICKLE hemoglobin gene from the previous page. Write the mRNA sequence below.

5. Translate the mRNA sequence into its corresponding amino acids. Use the chart of mRNA codons on the next page. Write each amino acid in the boxes below.

Amino Acid Sequence for SICKLE hemoglobin:

--	--	--	--	--

6. Record the charge of each amino acid on the line provided below each box. Refer to **Document 3: The Structural Formulas for the Amino Acids** for assistance.
7. Do they differ? If so how?
8. How do you think the differences affect protein function?

The Mystery Disease

A Closer Look at the Cause of Sickle Cell Anemia

Document 2:

A Chart of mRNA Codons and Their Corresponding Amino Acids

SECOND BASE OF CODON

		SECOND BASE OF CODON					
		U	C	A	G	3' End	
FIRST BASE OF CODON	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr Stop Stop	Cys Cys Stop Trp	U C A G	THIRD BASE OF CODON
	C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G	
	A	Ile Ile Ile Met(Start)	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G	
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G	

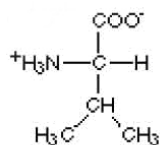
The Mystery Disease

A Closer Look at the Cause of Sickle Cell Anemia

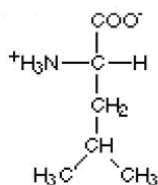
Document 3:

The Structural Formulas for the Amino Acids

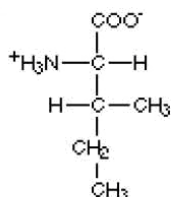
Amino acids with hydrophobic side groups:



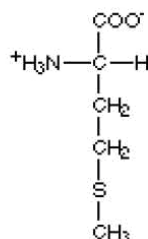
Valine
(val) V



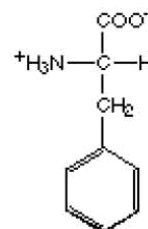
Leucine
(leu) L



Isoleucine
(ile) I

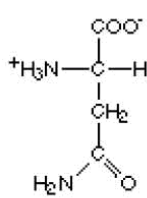


Methionine
(met) M

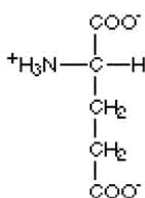


Phenylalanine
(phe) F

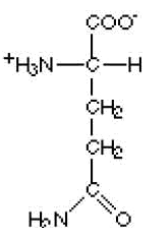
Amino acids with hydrophilic side groups:



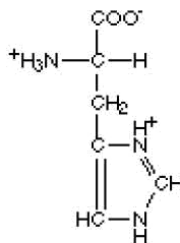
Asparagine
(asn) R



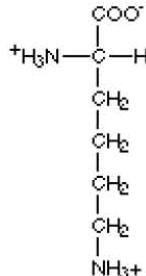
Glutamic acid
(glu) E



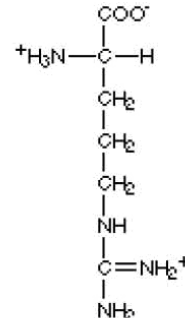
Glutamine
(gln) Q



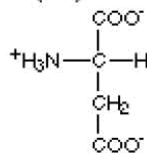
Histidine
(his) H



Lysine
(lys) K

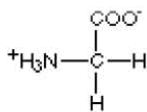


Arginine
(arg) N

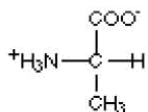


Aspartic acid
(asp) D

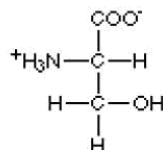
Amino acids that are in between:



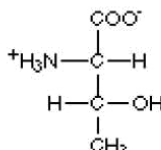
Glycine
(gly) G



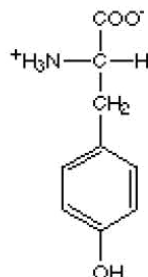
Alanine
(ala) A



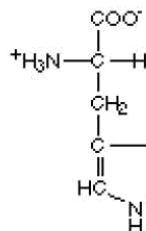
Serine
(ser) S



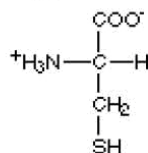
Threonine
(thr) T



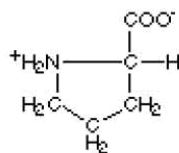
Tyrosine
(tyr) Y



Tryptophan
(trp) W



Cysteine
(cys) C



Proline
(pro) P

Patient Description Sheet

In 1904, a student from the West Indies came to a Chicago physician, Dr. James Herrick, with a puzzling condition. Below is a summary of some of the observations Dr. Herrick made. Your job is to learn more about this condition and to find out how the disease affects the body. Read the description below and underline the information that you think may provide important clues that will help you understand the disease.

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 his 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 have this condition. His uncle and his grandmother often had similar symptoms. His grandmother died a young woman. His parents do not have this condition.

James Herrick

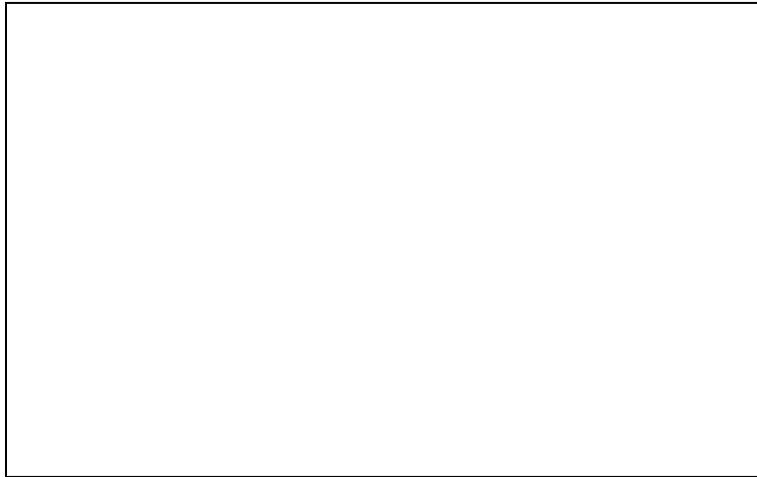
1. What symptoms do you find concerning?
2. Are the patient's various symptoms related to one another? If so, how? If not, explain why.
3. What would you do to further study this mystery disease?

The Mystery Disease

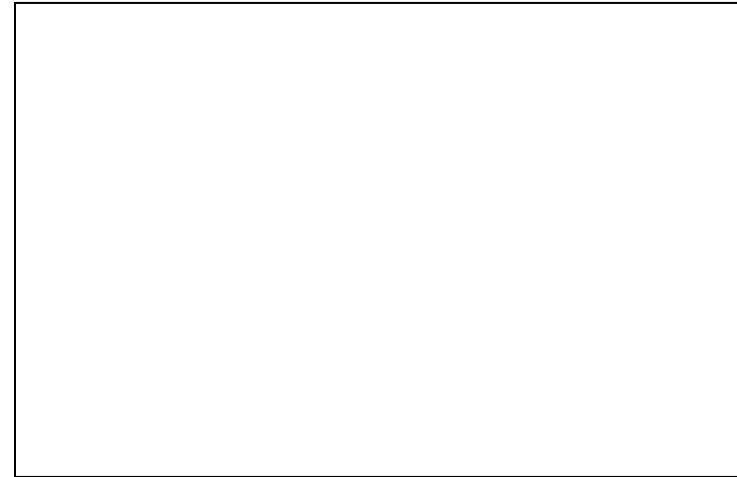
Name: _____

Histological Examination

Examine the slides of a normal blood smear versus that of the mystery patient. Draw what you see below.



Normal Blood



Mystery Patient's Blood

1. What differences do you see between the patient's blood and the normal blood?

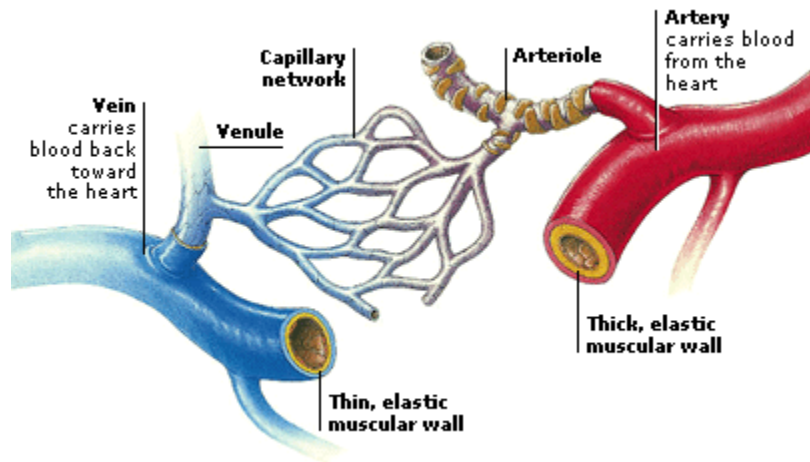
2. How might these changes affect a person's circulatory "health"?

Blood Flow Models

Background Information

There are three major types of **blood vessels**: arteries, capillaries and veins. **Arteries** are the blood vessels that carry blood away from the heart (see figure below). Arteries generally contain oxygenated blood.

Arteries become smaller the further they are from the heart and eventually form **arterioles**, which ultimately form branching structures called **capillaries**. The capillaries are small enough for diffusion and transport to occur across their membranes – so oxygen can be delivered to the cells, carbon dioxide can be taken up, waste picked up, nutrients transported, etc.



Capillaries eventually turn into larger structures called **venules**, which, in turn, become **veins**. Veins bring blood back to the heart (see figure above) and generally contain de-oxygenated blood.

Objective: You will explore how the morphological changes seen in the histology slides may affect the flow of blood using flasks of three different sizes and models of normal red blood cells and the mystery patient's red blood cells.

1. What do each of the different-sized flasks represent?
2. Explain how you will go about exploring the relationship between red blood cell morphology and blood flow in arteries, veins, and capillaries.
3. After conducting the activity, explain what you have discovered.

Pedigree of Mystery Patient (1923)

A family history reveals that the mystery patient has two brothers and three sisters, and although several of his siblings have minor symptoms, none of his siblings have problems nearly as severe as those experienced by the patient himself. His grandmother and one niece used to have similar severe symptoms to the patient. His grandmother died a young woman.

In 1923, researchers W. H. Taliaferro and J. G. Huck took blood samples from each of the mystery patient's surviving relatives to see how they compared to the sample of blood from the patient. They used a technique developed by V. Emmel in 1917. All blood samples were drawn and placed under airtight cover slips on slides for later analysis. This involved first placing a paraffin (wax) ring on a slide. Blood was taken from an individual and placed in the center of the ring. A cover-slide was then placed down onto the slide such that the blood was sandwiched between the cover slip and the slide. After 48 hours under these conditions, Taliaferro and Huck looked to see whether or not there were any changes in the blood cells.

On the following page, you will find a pedigree chart that summarizes the family genealogy and hematology data. When a person's blood slide showed blood characteristics (the odd-shaped red blood cells) similar to the mystery patient's, their respective square or circle was darkened. **Take caution – the pedigree only depicts whether or not the blood cells changed shape. It does not depict the differences in symptoms discussed above (e.g. severe, mild and none).**

1. Is this disease inherited? _____
 - a. If so, what type of inheritance accounts for the pattern seen in the pedigree?
What is your evidence?

 - b. If you do not think that the disease is inherited, what is your evidence?

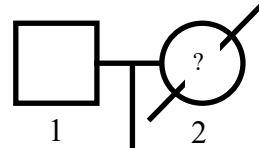
The Mystery Disease

Name: _____

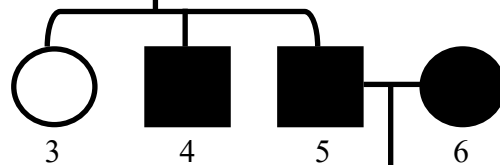
Pedigree of Mystery Patient (1923)

Generation

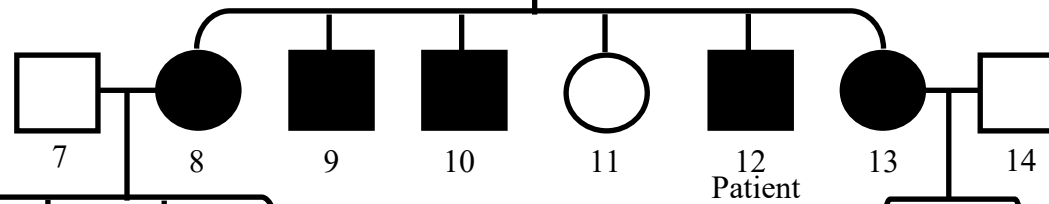
I



II



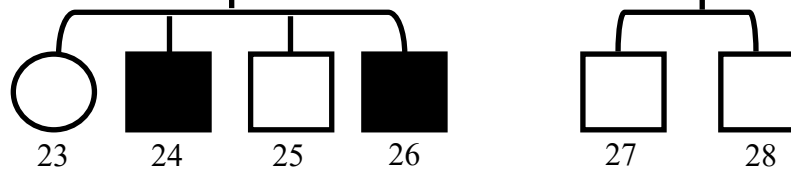
III



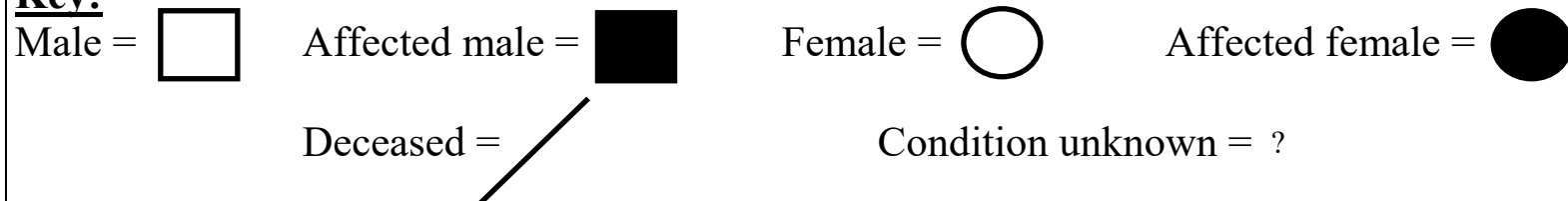
IV



V



Key:



In-vitro vs *In-vivo*

Researchers were puzzled that the blood of both the severe sufferers of the disease (like the mystery patient) and the mild sufferers changed shape under Emmel's 48-hour *in-vitro* test. Why was this occurring? Was there some change occurring in the blood with respect to the shape of the red blood cells that was not being caught by the *in-vitro* test? Read on to find out more about the difference between *in-vitro* and *in-vivo* tests....

BETWEEN BODY AND PETRI DISH

In-vitro and *in-vivo* are two Latin words used in biology to refer to different types of testing that are done in research. *In-vitro* is literally translated as "in glass", while *in-vivo* literally means "within the living".

Often, *in-vitro* tests or medical procedures are done as opposed to *in-vivo* tests for practical reasons. For example, "*in-vitro* fertilization", refers to the practice of injecting sperm into a viable ovum in a Petri dish and then surgically reintroducing the zygote into the uterus of the mother.

In-vivo tests, on the other hand, are done under conditions that either exactly or closely mimic those found within the body. For example, a common procedure that is used to determine if a person has a stomach ulcer involves a doctor sending a special, miniaturized camera down the patient's esophagus, through the cardiac sphincter, and into the stomach.

The only way to truly measure what is happening to the blood *in-vivo* is to somehow monitor what is going on while the blood is actually in the arteries and veins. As soon as blood is drawn away from the body (such as with a needle), an *in-vitro* environment has begun. The more quickly the blood is analyzed after it has been taken out of the body, the more accurate one can assume the results will be because the conditions more closely mimic those found within the blood's *in-vivo* state. The longer one waits to analyze the blood after it has been extracted, the more likely it will be that changes have occurred that may alter the results.

The blood contains living cells that carry on metabolic processes. When it is extracted from the body, the cells within the sample of blood will continue carrying out their metabolic processes as if they were still within the body. However, there is only a fixed amount of reactants, like oxygen and minerals, contained within the blood sample, which cannot be replenished, so the cells will use up their resources. This is one of the reasons why blood samples are placed on ice following the removal from the body. The cold temperatures slow the metabolic processes of the cells, which helps to maintain a blood sample in as close to an *in-vivo* state as possible.

Further Pedigree Analysis (1947)

In 1947, further research was done with the mystery patient's family to try to better understand the mechanism of inheritance of the disease. Whereas the previous blood analysis used *in-vitro* results (blood that had remained under a cover slip for a couple of days), a new technique was employed that could analyze *in-vivo* samples. This new technique was based on experiments conducted by I. Sherman, a physician from Johns Hopkins University, in 1940.

A family member's blood was drawn from their vein, put under a cover slip that already maintained environmental conditions similar to the person's veins (pressure, acidity, etc.), and examined using a microscope. What resulted from this research is depicted in the pedigree contained on the next page.

Using the *in-vivo* technique, scientists were able to distinguish between those individuals whose red blood cells changed shape in the veins versus those whose shape remained "normal" while in the veins. Note that the symptoms have not changed from the earlier pedigree - those surviving members (e.g. the patient's brother) who suffered milder symptoms in the previous sample still suffer from milder symptoms.

1. Why is an *in-vivo* test preferable?

2. Given this pedigree, do you believe that genetics still plays a role in the transmission of the disease? If so, how?

3. How does the technique used to determine this pedigree differ from the technique used to determine the previous pedigree?

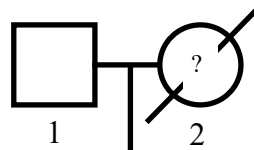
4. Was the theory that you developed based on the 1923 pedigree data "wrong"? Explain.

The Mystery Disease

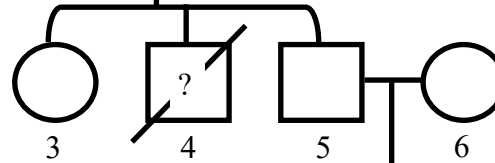
Further Pedigree Analysis (1947)

Generation

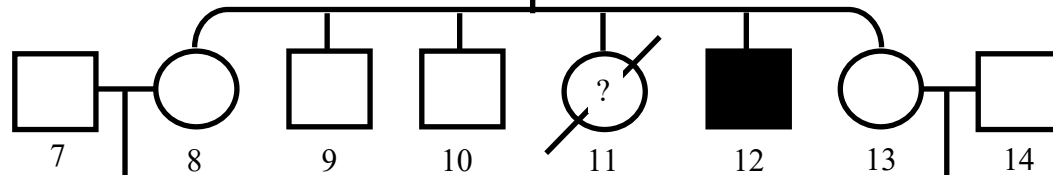
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II



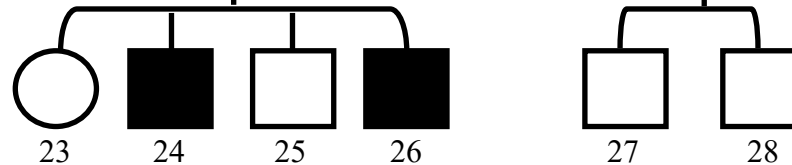
III




IV





V




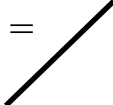
Key:

Male = 

Affected male = 

Female = 

Affected female = 

Deceased = 

Condition unknown = ?

Why Is There Such a High Prevalence of the Mystery Disease?

Student Challenge:

For this activity, your challenge is to come up with an explanation for why a seemingly deleterious allele (the allele for this mystery disease) is maintained in a population when, normally, deleterious alleles are removed from a population over time.

You will use some of the data that scientists used during the 1940s and 1950s to come up with your own explanation to account for the unusually high frequencies of this allele. The data are found on the following pages, and they come from Uganda, Africa.

Specifically, your goals will be to attempt to come up with theories to explain why:

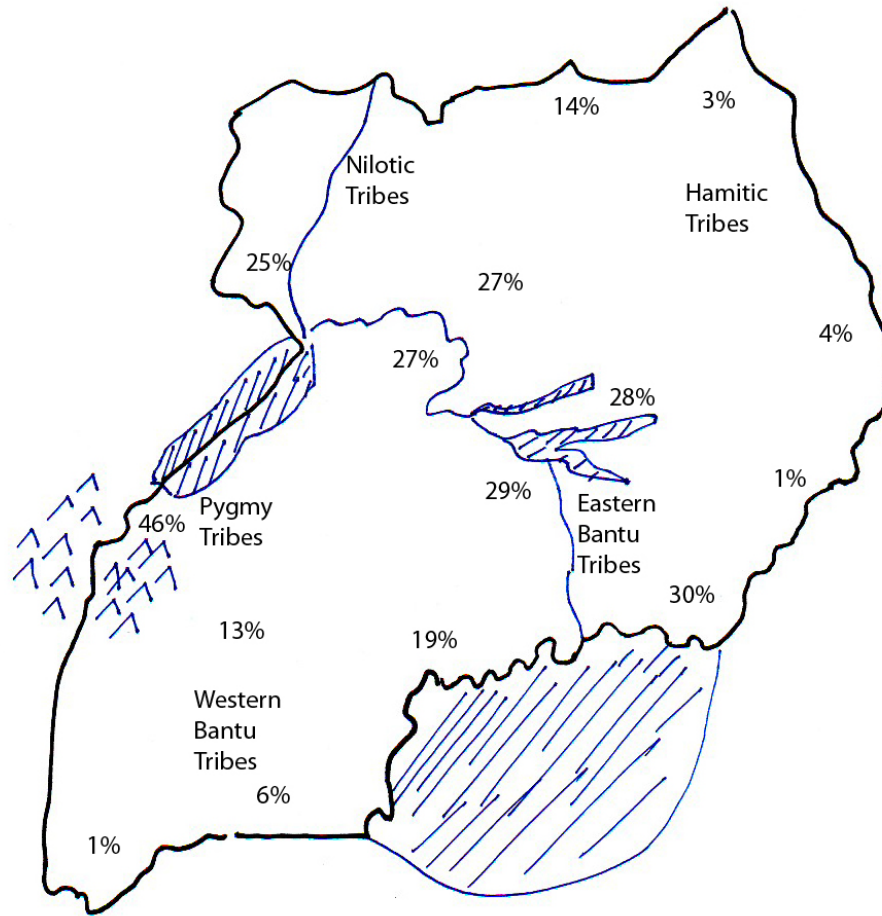
- a) There appear to be a high number (frequency) of carriers of the disease in certain locations.
- b) Why there is such a mixture of high frequencies and low frequencies across the country.

Before you begin, answer the following question:

1. As scientists, you all have access to the same data for this problem. Do you think that you will all come up with the same explanation for the unusually high frequencies of this allele? Why or why not?

Why Is There Such a High Prevalence of the Mystery Disease?

Uganda Tribes and Allele Frequencies



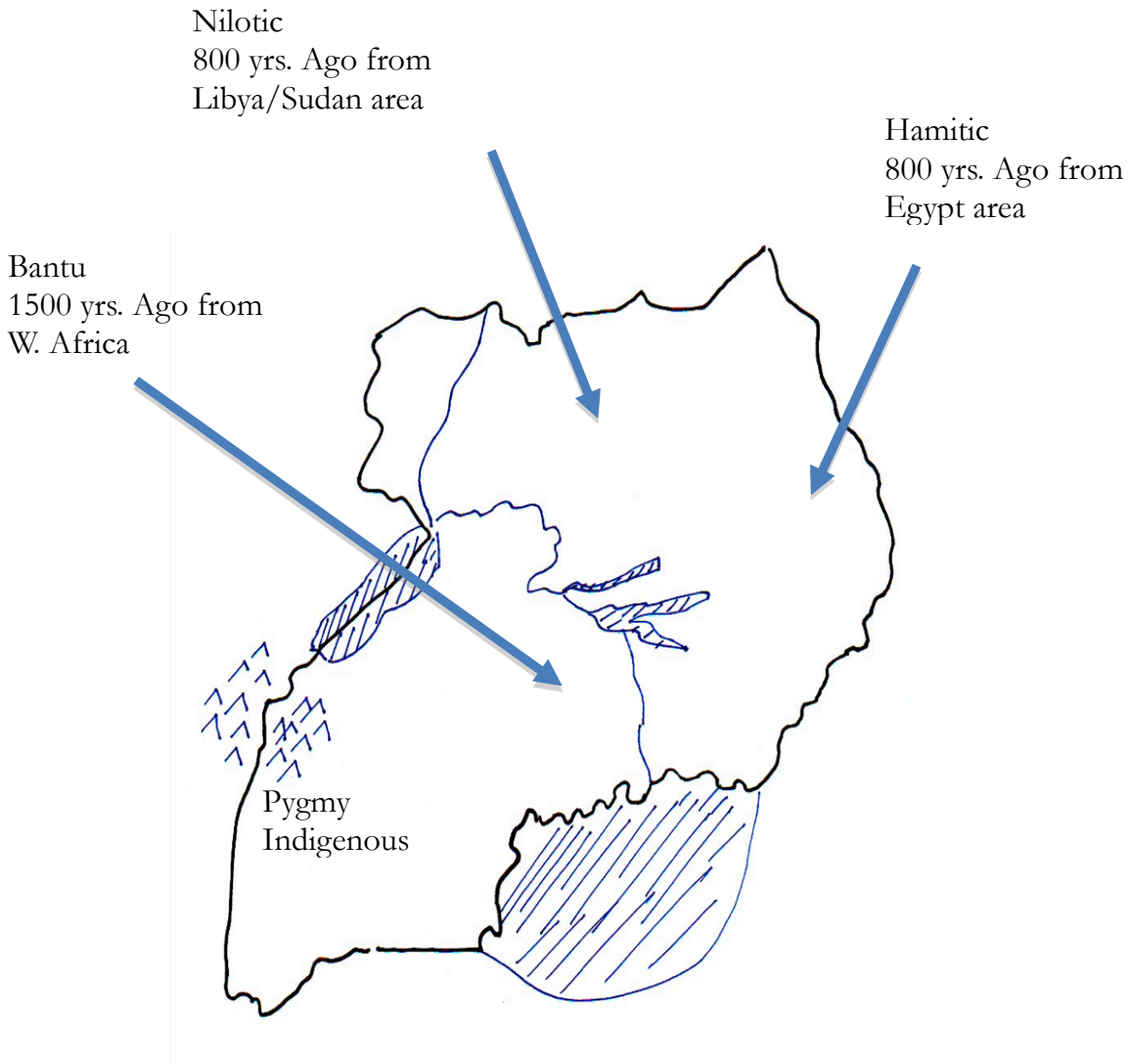
Data adapted from:
 Herrick, A. (1969). Area Handbook for Uganda, U.S. Gov't. Printing Office, Washington, pp. 79-81.
 Allison, A. C. (1954).
 Lehmann, H. (1953).

Tribal Group	Allele Frequency	Between Group Contact*
Bantu (Eastern)	High	With Nilotic
Bantu (Western)	Moderate	None
Hamitic	Low	None
Nilotic	High	With Eastern Bantu
Pygmy	Very High	None

*Contact means the amount of immigration, emigration, and intermarriage that occurred with potential neighbors.

Why Is There Such a High Prevalence of the Mystery Disease?

Uganda Tribal Group Immigration Data

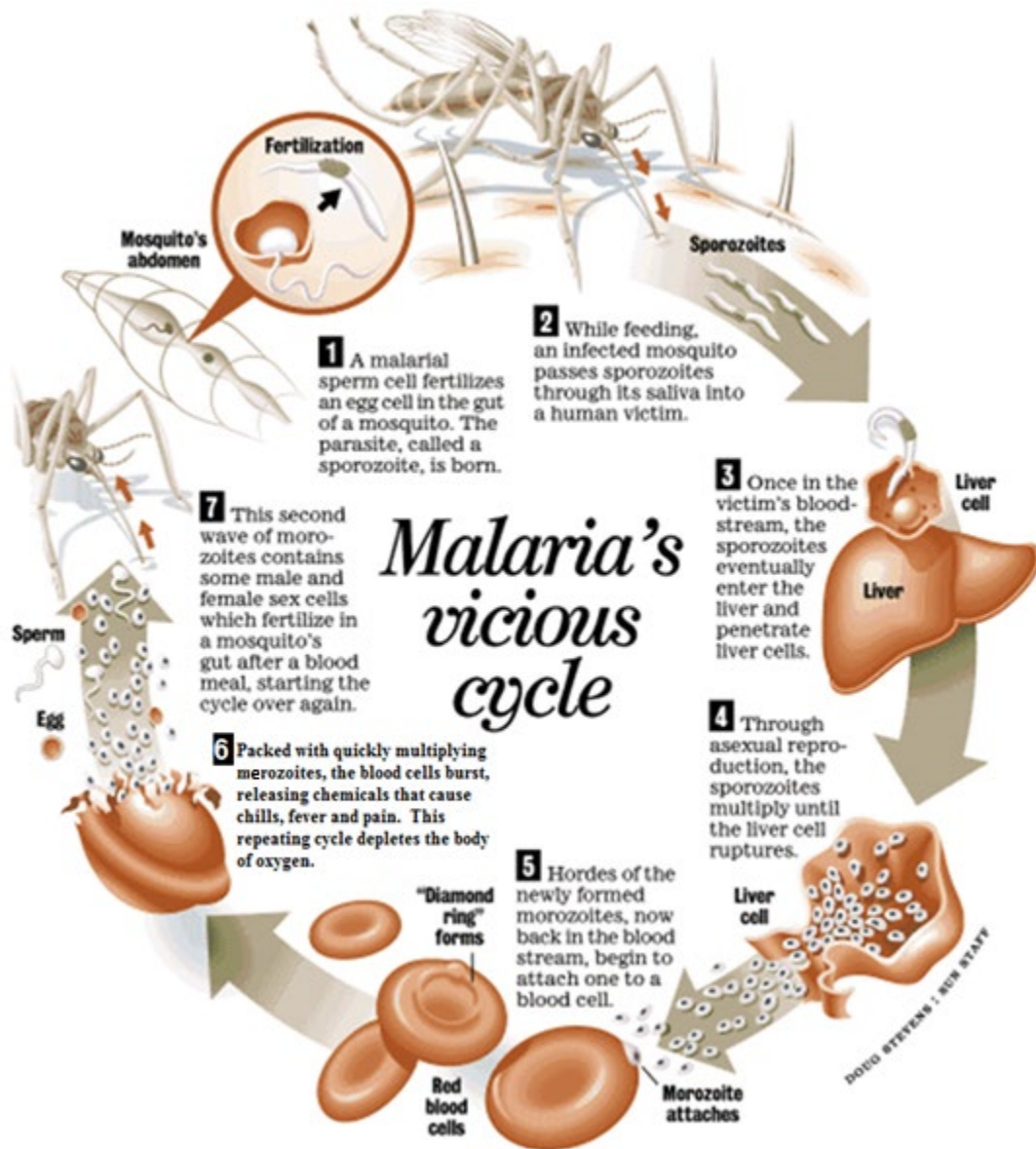


Why Is There Such a High Prevalence of the Mystery Disease?

Malaria's Vicious Cycle

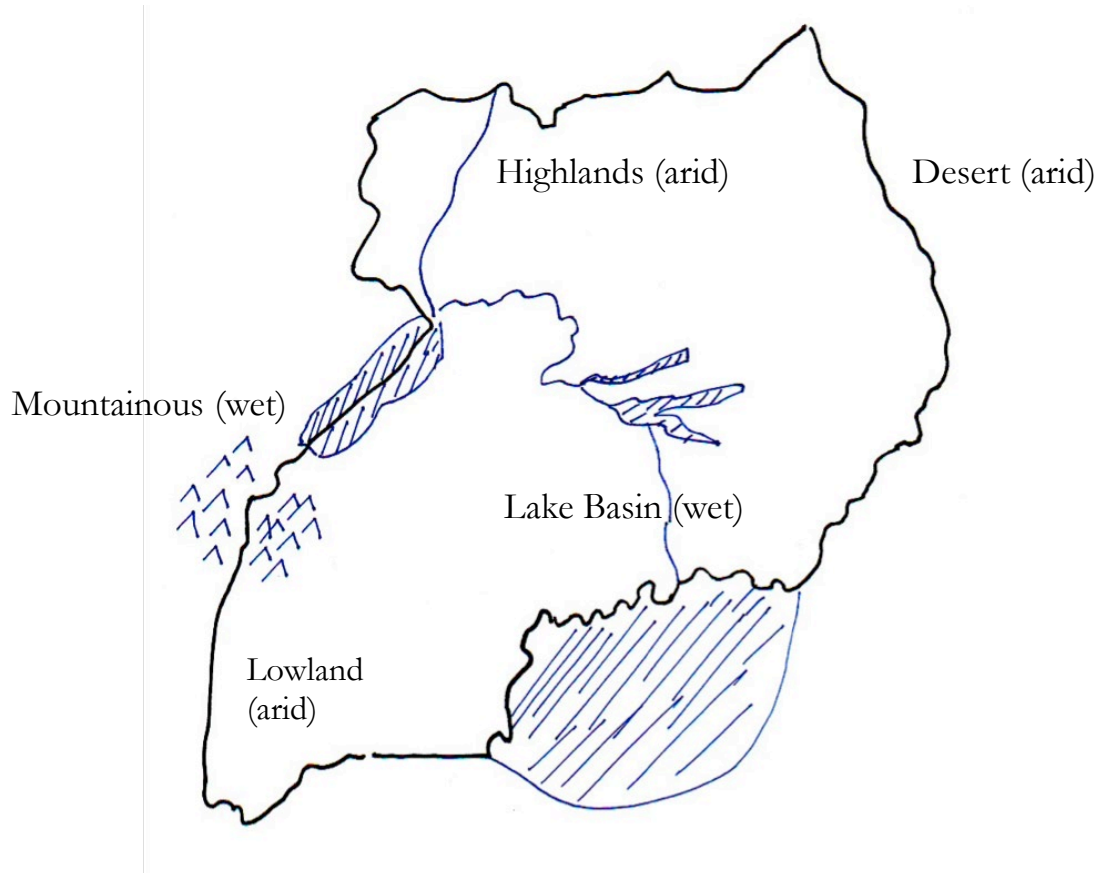
While medical researchers were performing blood tests on various tribal groups in Uganda, they noticed that certain infectious diseases, such as malaria, were prevalent in the area. So, the researchers also qualified and quantified the degree of severity of malaria across Uganda.

Below is information regarding the malarial parasite's life cycle and on the following pages are data regarding the incidence of malaria across Uganda.



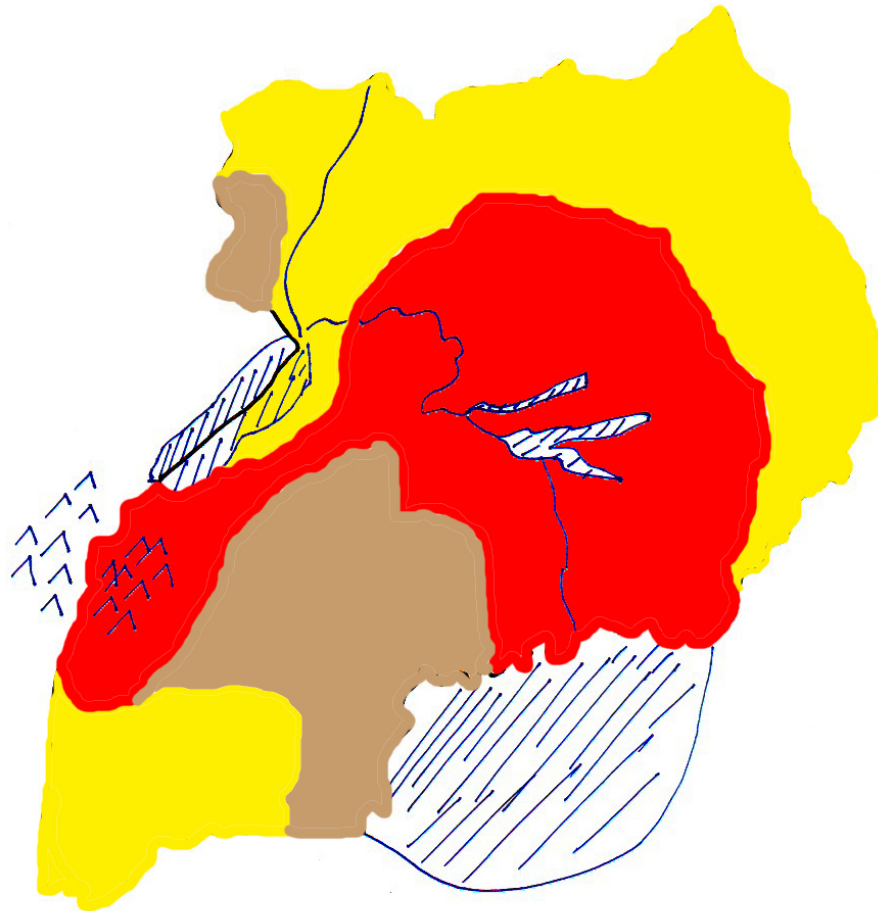
Why Is There Such a High Prevalence of the Mystery Disease?

Weather in Uganda



Why Is There Such a High Prevalence of the Mystery Disease?

Exposure to Malaria in Uganda

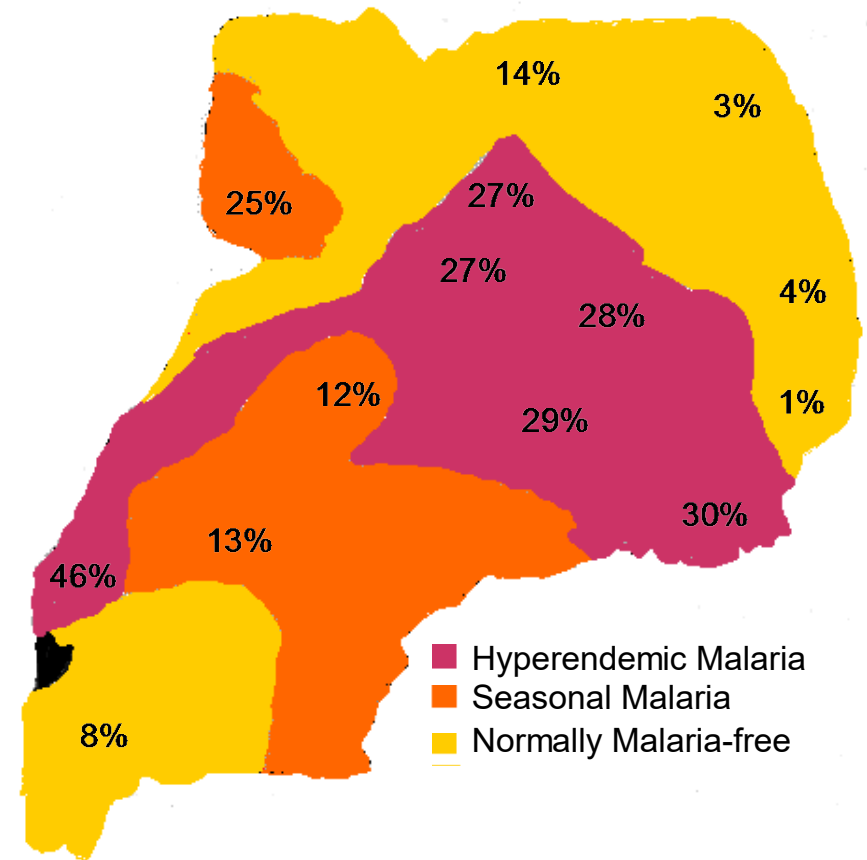
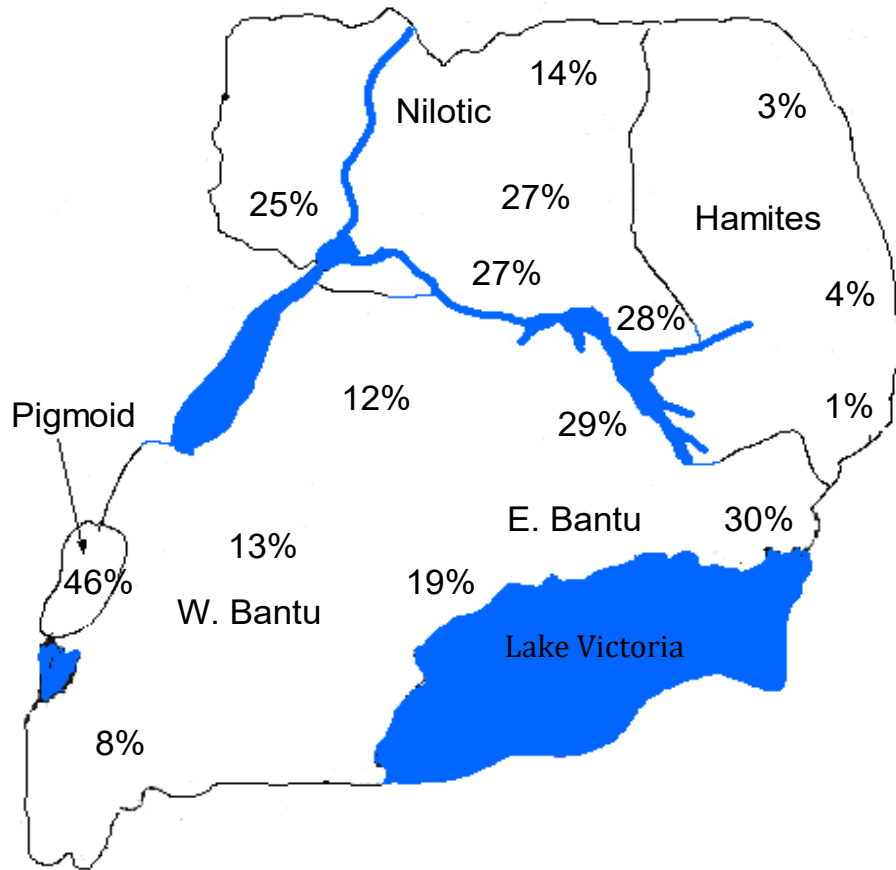


- Normally malaria free
- Seasonal malaria (periods of exposure followed by relief)
- Hyperendemic malaria (continual exposure)

Why Is There Such a High Prevalence of the Mystery Disease?

1954: Heterozygote Advantage

Allison's Research



Why Is There Such a High Prevalence of the Mystery Disease?

Anthony C. Allison's Research

Anthony C. Allison, a medical researcher in the early 1950's, was interested in conducting further analyses on the apparent correlation between the seemingly high frequencies of the mystery disease and the presence of malaria in Uganda. Allison drew blood samples from Ugandan children to use for his research on the mystery disease. He analyzed the blood of each child to determine whether they were a carrier for the disease or had a normal genotype, and for the presence of the malarial parasite, *Plasmodium falciparum*, including its density in the red blood cells.

Genetic Disposition	Total Number of Children Examined	% Children with <i>P. falciparum</i>	Parasite Density Index
Normal (“+ / +”)	247	46	5.9
Carrier (“+ / -”)	43	28	4

Note: Parasite Density Index = a general measure of the amount of malarial parasites in the red blood cells per volume of blood.

(Adapted from: Allison, A. C. (1954) and Raper, A. (1959)).

2. Do you think these new data shed any light on why there are high versus low frequencies of the mystery disease in various areas of Uganda? Explain.

3. Do you find it troubling that you are basing your explanations for the seemingly high frequencies of the mystery disease on data collected using observational methods rather than controlled experiments? Why or why not?

Why Is There Such a High Prevalence of the Mystery Disease?

Data Analysis and Conclusions:

To help you with the following questions, fill in the following chart based on what you've learned about the mystery disease:

Genotype	+ / +	+ / -	- / -
Red Blood Cell Shape?			
Advantage?			
Disadvantage?			

- Both the earlier in-vitro blood test and the later in-vivo blood test that you learned about measured the presence of structural changes in the red blood cells under low oxygen environments. What could be affected within the red blood cells to cause these structural changes?

- Based on your answer to question #4, what are possible differences between the blood of a normal person, a carrier, and the mystery patient?

Why Is There Such a High Prevalence of the Mystery Disease?

6. Why do you suppose there is an increased resistance to malaria in those who are heterozygous for the mystery disease allele?

7. Do you think experiments are necessary for knowledge to develop in science?

8. Has your hypothesis to explain the frequencies changed? If so, what caused it to change? If not, why not?

Laboratory Protocol

Protein gel electrophoresis is a way to test for structural differences in the hemoglobin found in normal people, people who are heterozygous for the “mystery disease”, and people who have the full-blown version of the disease. Dr. Linus Pauling, a famous scientist, was the first to run gel electrophoresis on hemoglobin samples in 1949 in an attempt to find out more about what might be wrong with the structure of the hemoglobin in people with the “mystery disease”.

Today, you are going to be testing three different samples of hemoglobin, one from a person with normal hemoglobin, one from a person with sickle cell hemoglobin, and one from a mystery patient with symptoms similar to those of Dr. Herrick’s mystery patient. Your challenge is to determine if your patient has normal hemoglobin, sickle cell hemoglobin, or is possibly a carrier for the disease.

Loading Samples

1. Your teacher will assign you a gel and a set of well numbers. Write your well numbers in the table:

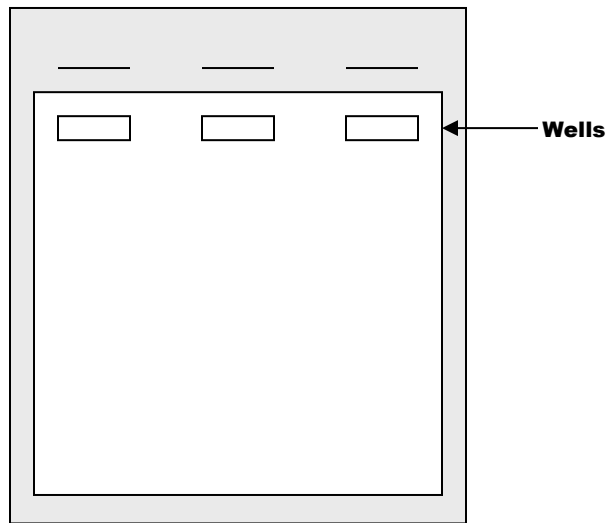
Gel Tray Number:	
Samples	Well Numbers
Normal Hemoglobin (A)	
Sickle Cell Hemoglobin (S)	
Patient’s Hemoglobin (P)	

2. Load **20 µl** of each of the normal hemoglobin control “**A**”, the sickle cell hemoglobin control “**S**”, and the patient’s hemoglobin sample “**P**” into your assigned wells of the gel.
Use a new pipette tip between the different samples to prevent contamination.
3. Once all samples are loaded into the gel, your teacher will place the gels in the electrophoresis box, pour electrophoresis buffer into the gel box, and cover it with the lid. Your teacher will hook the box up to a power supply and start running the gel at 200 volts for 15 minutes.

Sample Analysis

4. Observe the banding patterns on your gel. Do you see a difference between the normal and sickle cell hemoglobin controls? Compare the patient’s hemoglobin to the normal and sickle cell controls; which one is it similar to?

5. Draw what you see on your gel below. Be sure to label the wells with sample names, label both the positive and negative electrodes, and draw the direction of migration of the samples.

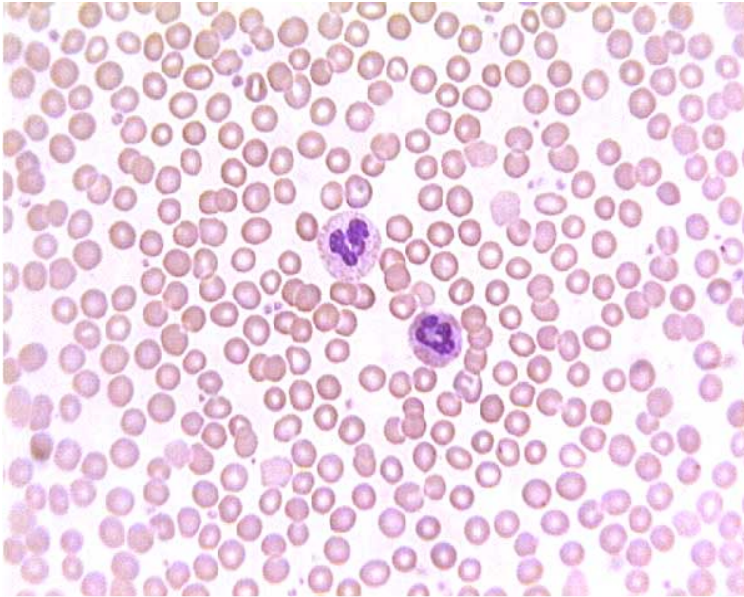


6. What is the function of the agarose gel?
7. Why is the gel in electrophoresis buffer?
8. What are the charges of normal and sickle cell hemoglobin? Which electrode on the gel box will the hemoglobin protein move towards?
9. What must you be careful of when loading the samples into the wells of the gel?
10. Even though the hemoglobin in the normal and sickle controls look very similar in the tubes how do they look different in the gel (talk about migration of bands, not colors)?
11. What is your diagnosis for your patient?
12. 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.

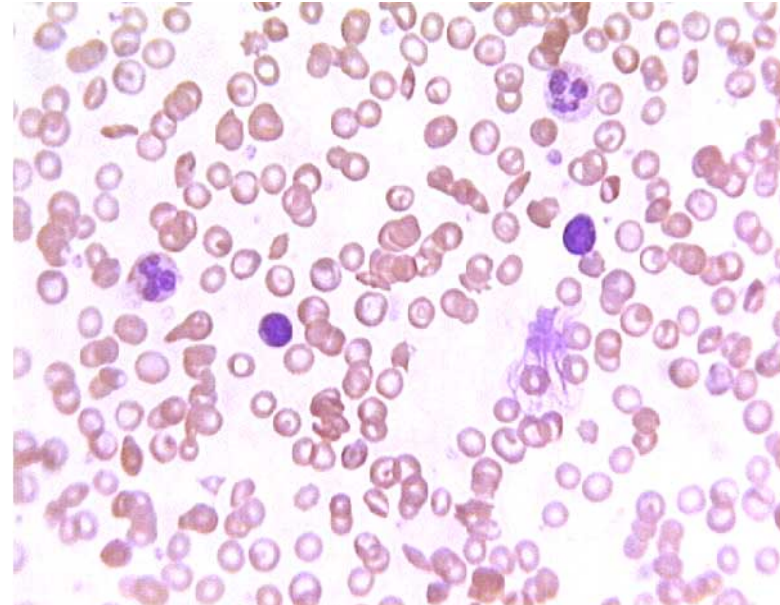
The Mystery Disease

Name: _____

Histological Examination - Alternate



Normal Blood



Mystery Patient's Blood

1. What differences do you see between the patient's blood and the normal blood?
2. How might these changes affect a person's circulatory "health"?
3. What further information might be helpful in exploring these questions?