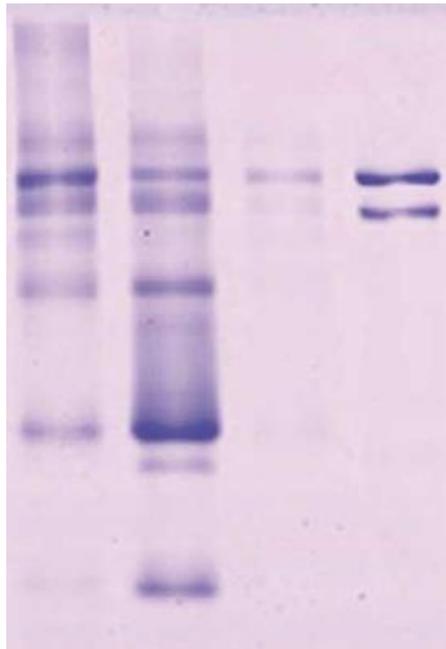


ANSWERING HEMOGLOBIN ELECTROPHORESIS QUESTIONS ON THE ASCP EXAM

Sohail Ahmed

Electrophoresis is a wonderful procedure used to separate an unknown into its constituent molecules.

The distinctive pattern visualized (depending on the media), serves as a “fingerprint” of the unknown you are attempting to identify.



Electrophoresis is used in both scientific research, and in daily testing 🙄

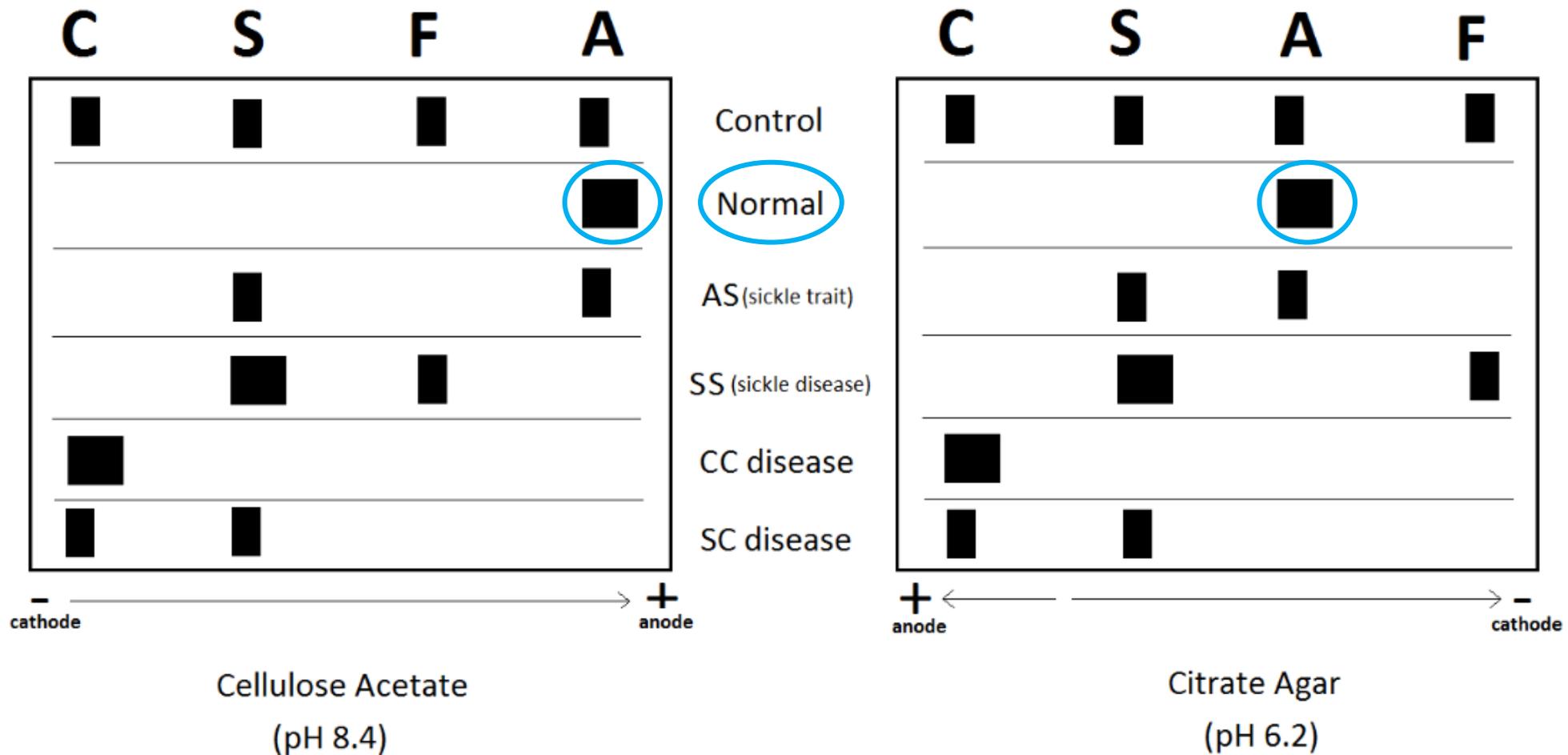
The concept is very important and I urge you to read about it on your own



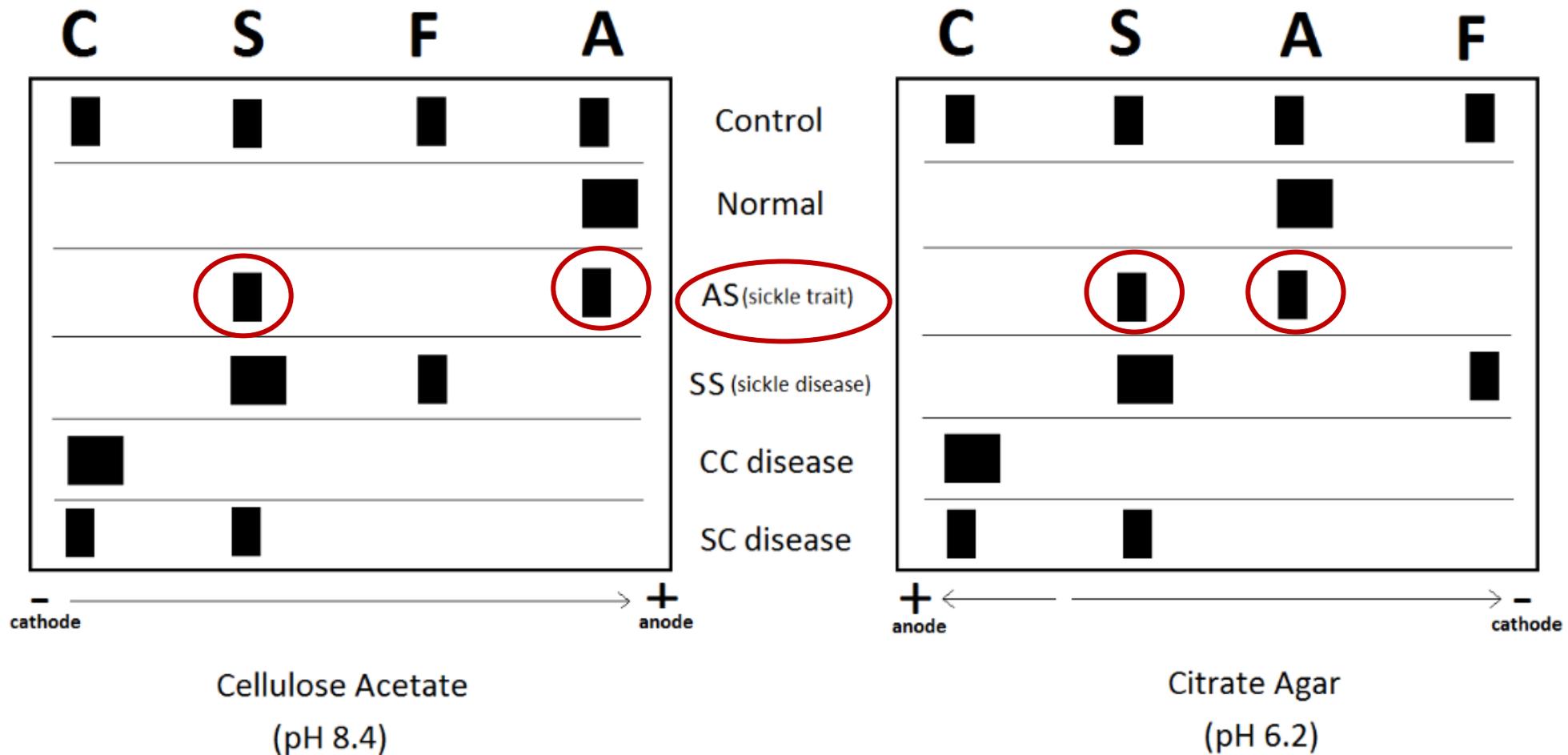
HOWEVER, we can forego any extensive explanation here! The following slides contain the most relevant information you might want to know for the ASCP exam.



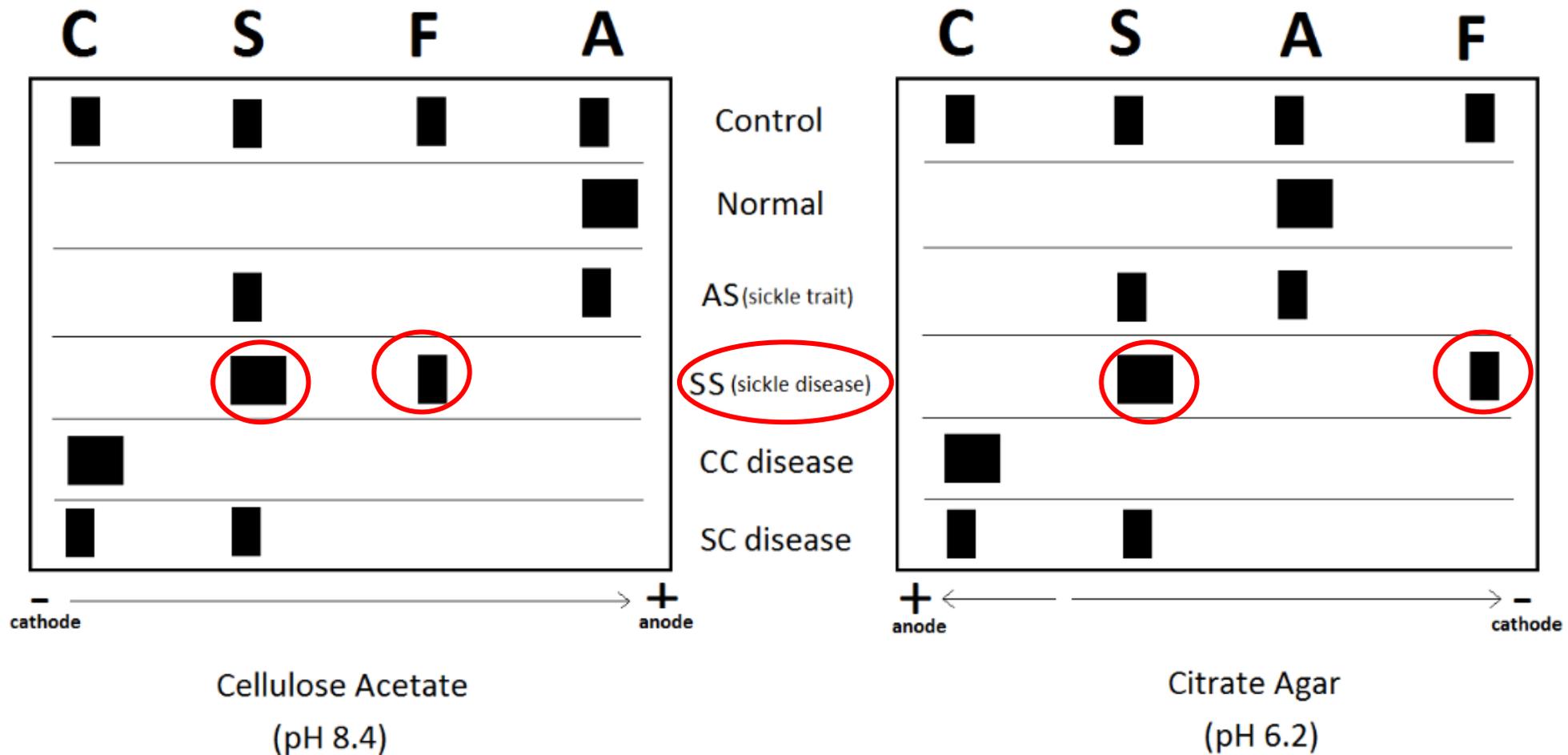
Take a look at the next slide. Don't memorize it! Just look at it 



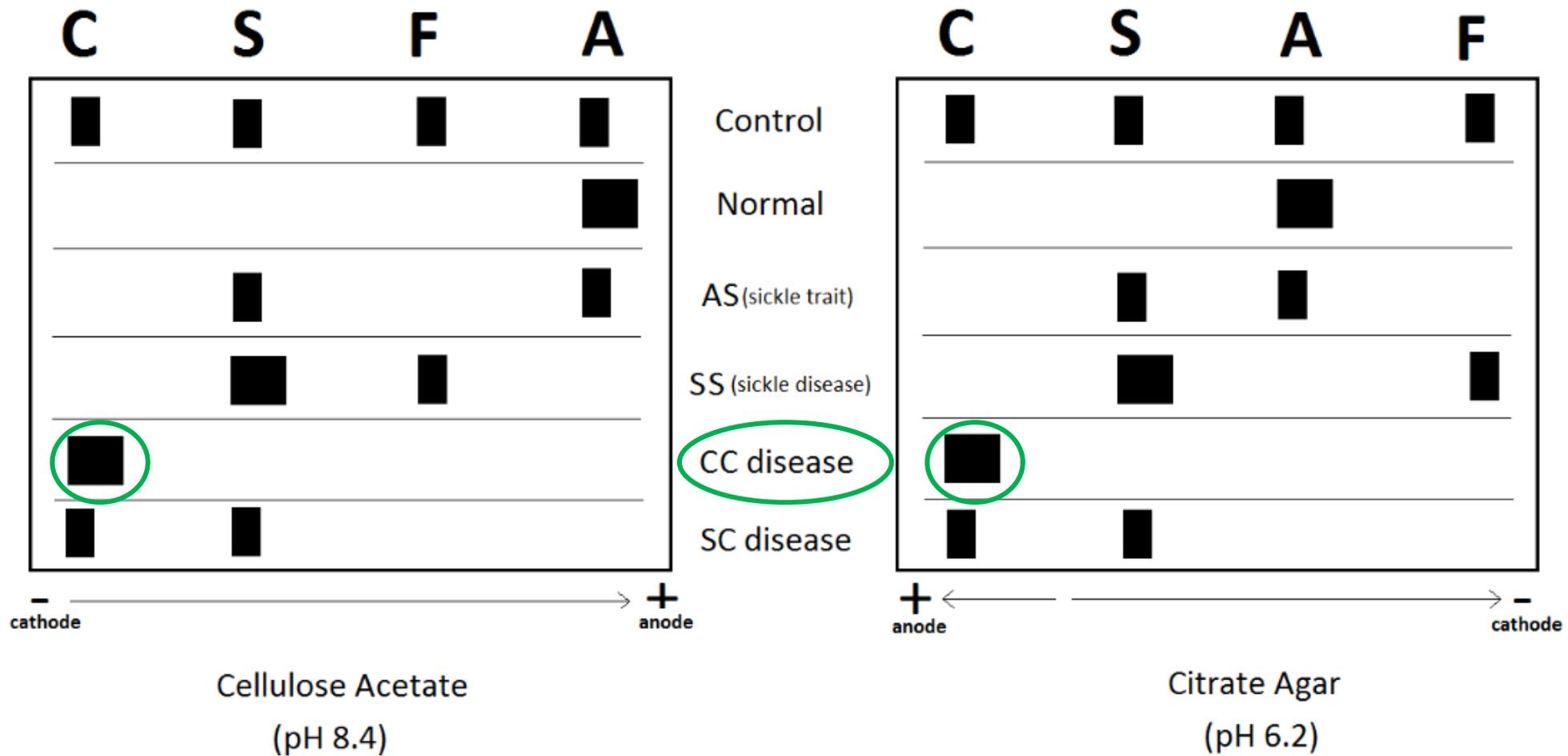
Normally, 95% of our hemoglobin is Hgb A. That is why a person with normal hemoglobin will produce one fat band at the Hgb A position. Take a look at the diagram. Do you see the fat band at the Hgb A position in both instances?



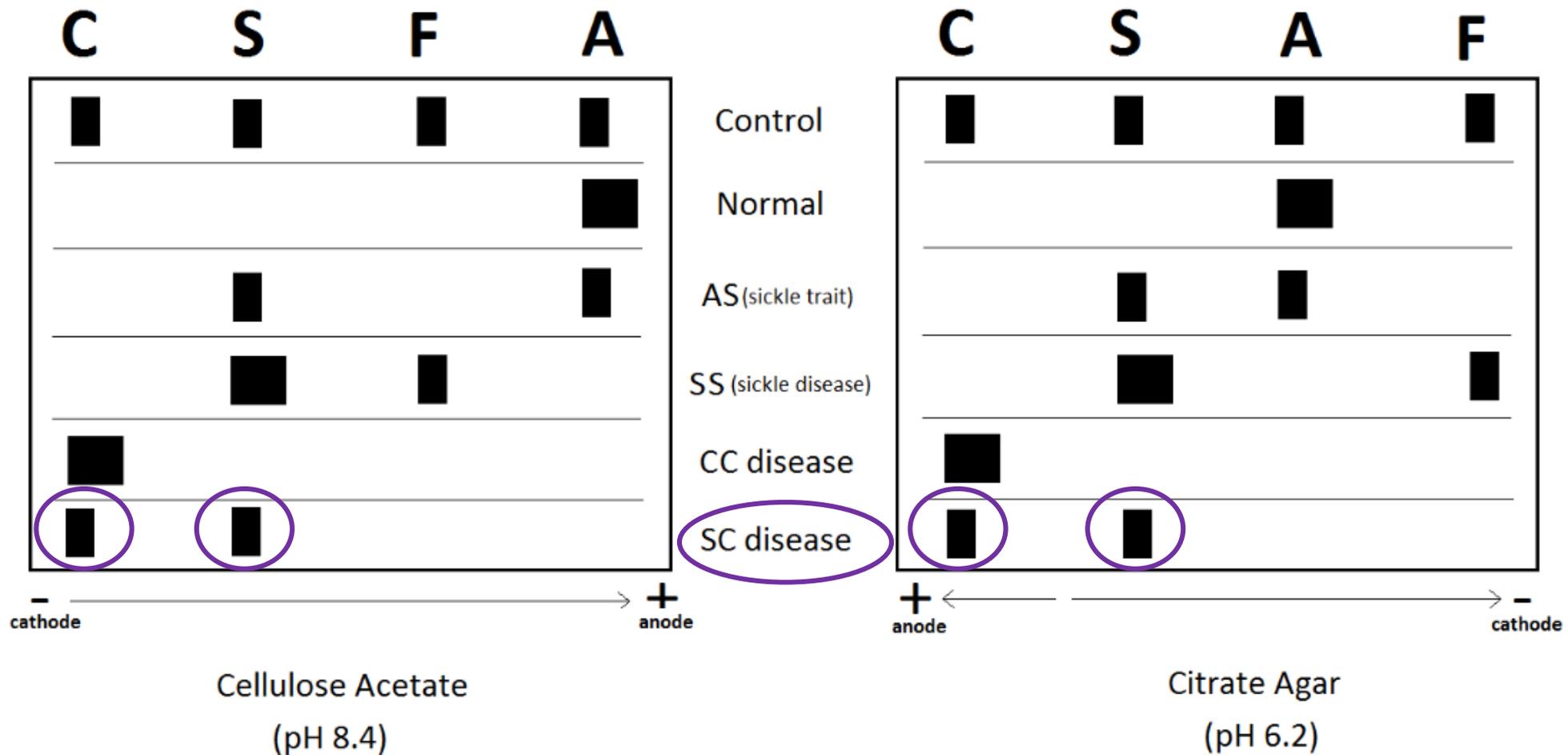
Folks with sickle cell trait (AS) are heterozygous; they have one gene for Hgb A and one for Hgb S. Clearly, such a person ought to demonstrate equal sized bands at the A and S positions. Do you see that in both diagrams above?



Folks with sickle cell disease (SS) have two sickle cell genes, and will thus produce a great deal of Hgb S. They will demonstrate one fat band at the S position. These folks also demonstrate a very thin band at the Hgb F position. Can you see this in both diagrams above?



Those with hemoglobin C disease (CC) have two genes for Hgb C. Thus, folks like this would produce a great deal of Hgb C. Do you see that in both diagrams above?



Finally, folks with hemoglobin SC disease are heterozygous; they have one gene producing Hgb S and one gene producing Hgb C. Such an individual will demonstrate equal sized bands at the S and C positions. Take a look at the diagrams above and confirm.

That was a lot of information. Here is what I would actually memorize:

-CSFA +
pH 8.4

+CSAF -
pH 6.2

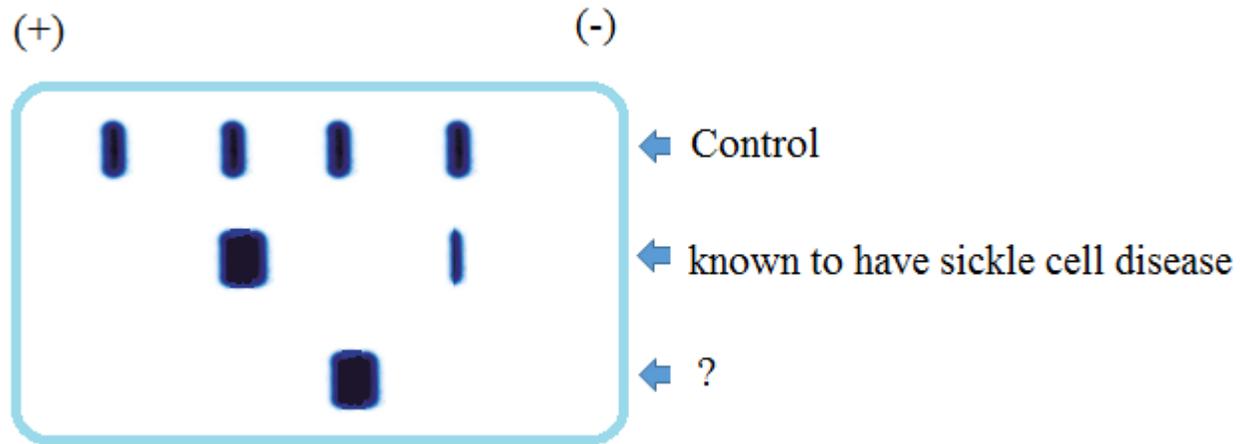
Whenever you come across a question dealing with hemoglobin electrophoresis, recall the above and you should be able to answer the question! We will try a few questions in the following slides.

**BEFORE YOU GO ON TO THE NEXT SLIDE, MAKE SURE YOU HAVE
MEMORIZED THE NOTATION ABOVE!**

QUESTIONS & ANSWERS

Question 1

What do you suspect here?



- A) Patient with Sickle Cell Disease (SS)
- B) Patient with Hemoglobin SC Disease (SC)
- C) Patient with Hemoglobin C Disease (CC)
- D) Patient with normal hemoglobin

Answer 1

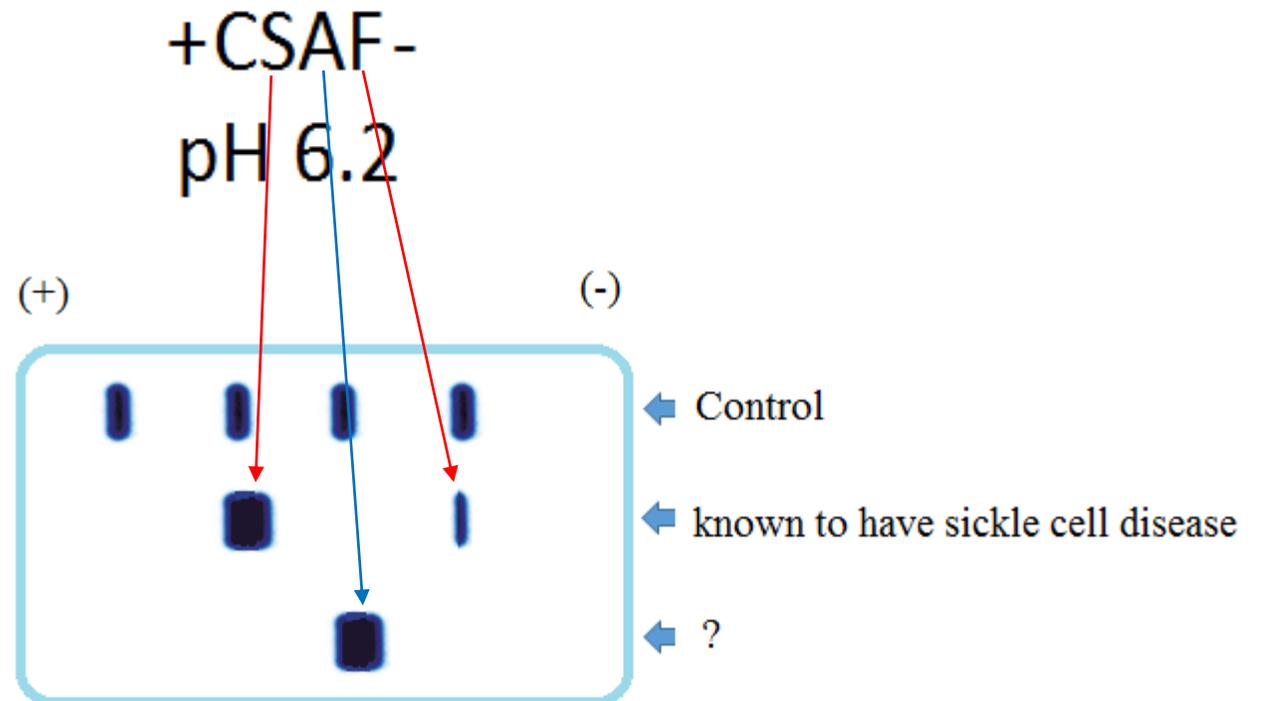
D) Patient with normal hemoglobin

>Recall the picture you've memorized. The patient with sickle cell disease is in the second row. Based on that, we know where Hgb F and Hgb S are located.

>Also note the location of the anode (+) and the cathode (-). Hgb F has separated out to a position adjacent to the cathode. In the diagram you memorized, Hgb F is adjacent to the cathode when electrophoresis is run on citrate agar (pH 6.2).

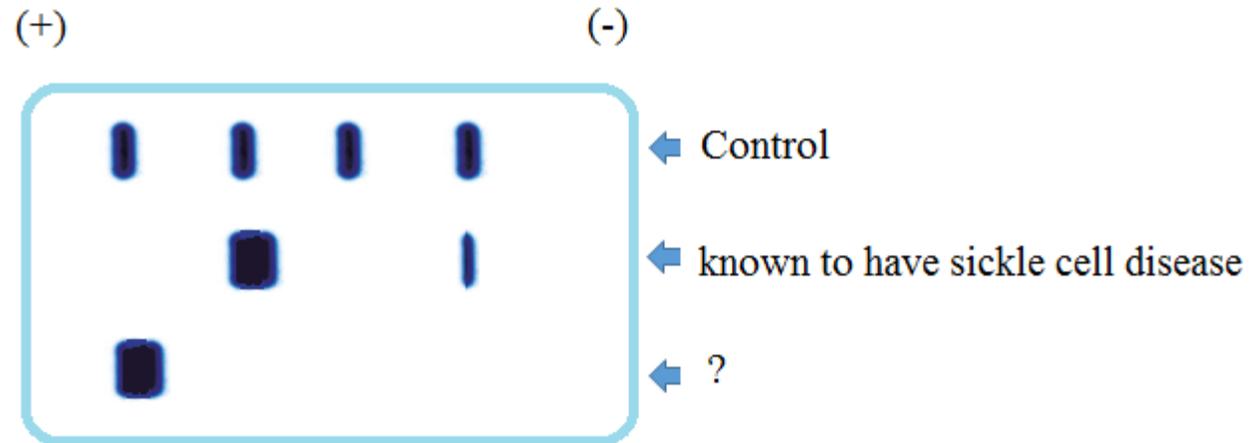
>The unknown in the last row is one large band which is located where Hgb A would be located at pH 6.2. Clearly, the unknown in the last row represents a patient with normal hemoglobin.

- CSFA +
pH 8.4



Question 2

What do you suspect here?



- A) Patient with Sickle Cell Disease (SS)
- B) Patient with Hemoglobin SC Disease (SC)
- C) Patient with Hemoglobin C Disease (CC)
- D) Patient with normal hemoglobin

Answer 2

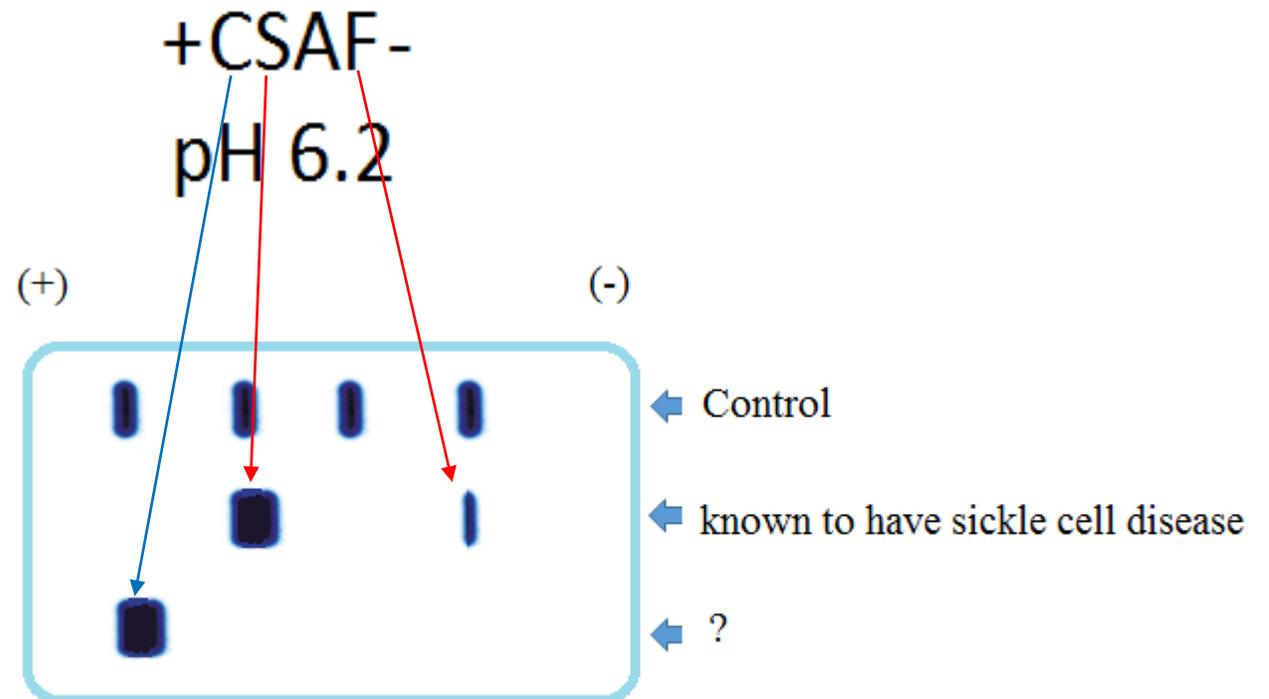
C) Patient with Hemoglobin C Disease (CC)

>Recall the picture you've memorized. The patient with sickle cell disease is in the second row. Based on that, we know where Hgb F and Hgb S are located.

>Also note the location of the anode (+) and the cathode (-). Hgb F has separated out to a position adjacent to the cathode. In the diagram you memorized, Hgb F is adjacent to the cathode when electrophoresis is run on citrate agar (pH 6.2).

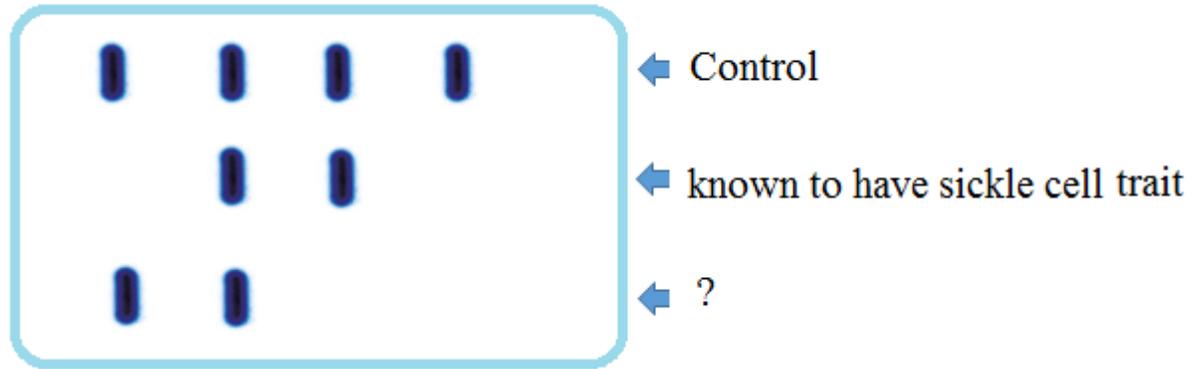
>The unknown in the last row is one large band located adjacent to the anode (+), which is where Hgb C would be located at pH 6.2. **The answer is C, a patient with Hemoglobin C Disease.**

- CSFA +
pH 8.4



Question 3

What do you suspect here?



- A) Patient with Sickle Cell Disease (SS)
- B) Patient with Hemoglobin SC Disease (SC)
- C) Patient with Hemoglobin C Disease (CC)
- D) Patient with normal hemoglobin

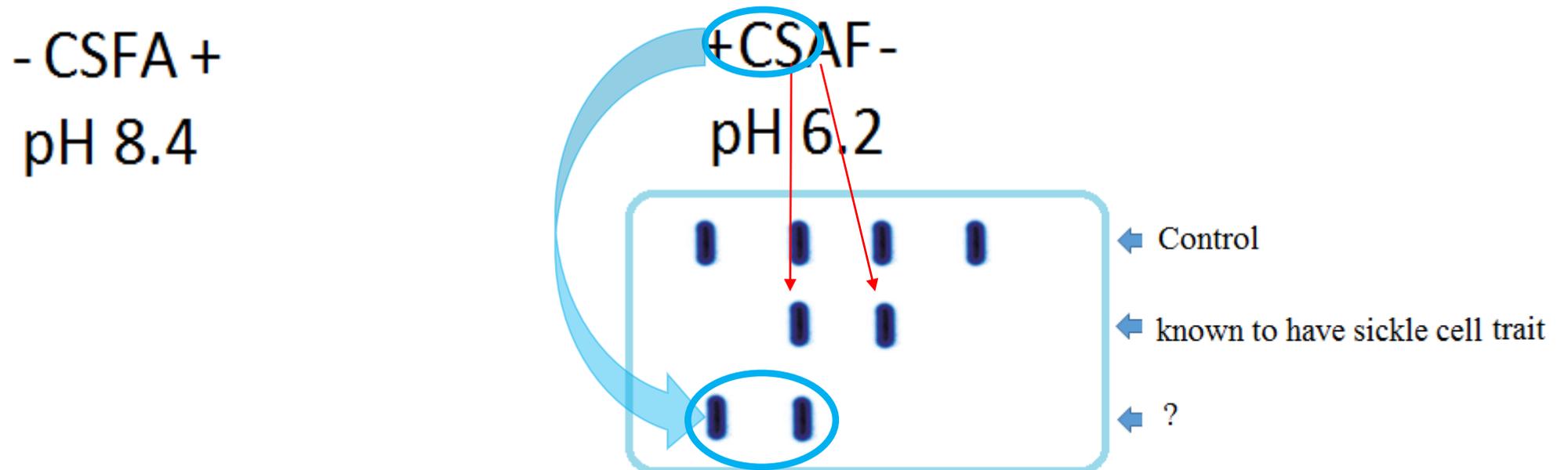
Answer 3

B) Patient with Hemoglobin SC Disease (SC)

>Choices C & D are eliminated because they should demonstrate only one band and the unknown here demonstrates two bands.

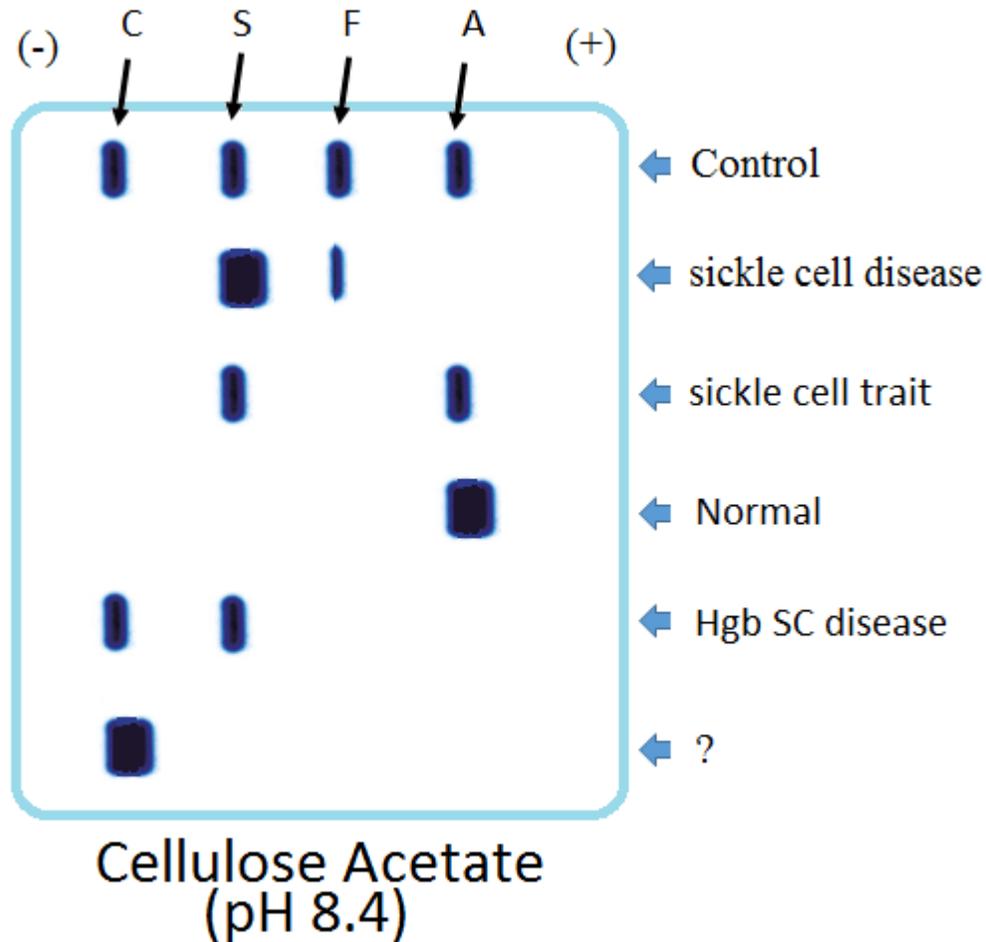
>Based on the pattern demonstrated by the known patient with sickle cell trait in the second row (two equal sized bands located adjacent to each other), we can determine that this electrophoresis is run on citrate agar (pH 6.2).

>We can't be certain of the locations of the anode or cathode because this diagram could simply be flipped horizontally. Then how can we decide between choices A and B? At pH 6.2, choice A would demonstrate two unequal sized bands located apart from each other. **Choice B would demonstrate two equal sized bands located adjacent to each other on one extreme end of the medium.** The answer is choice B, Hemoglobin SC Disease.



Question 4

A 28 year old patient presents with mild, chronic anemia. This young man has consistently demonstrated a low hemoglobin count for the 10 years he has been coming to this hospital for routine annual physicals. This has not proven to be a clinical problem but he is currently very concerned and his physician wishes to placate him. After ruling out other possibilities such as bleeding and poor nutrition, hemoglobin electrophoresis is performed. Based on the patients result (patient result in “?” lane) what problem is suspected?

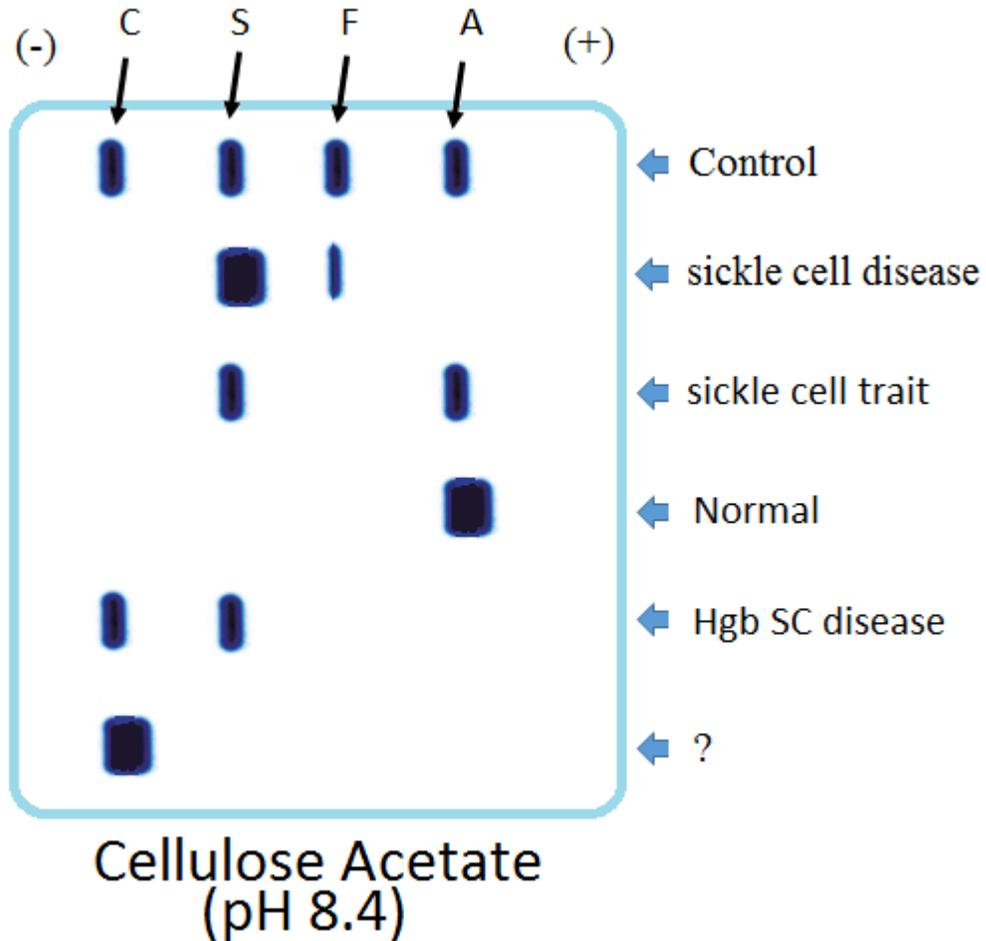


- A) Sickle Cell Disease (SS)
- B) Hemoglobin SC Disease (SC)
- C) Hemoglobin C Disease (CC)
- D) Sickle Cell Trait (AS)

Answer 4

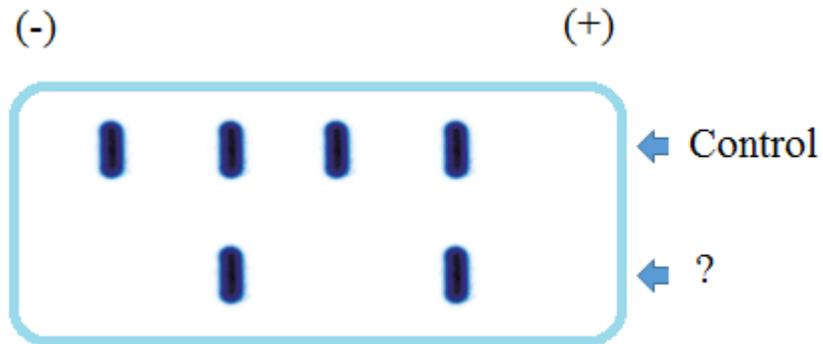
C) Hemoglobin C Disease (CC)

We can easily eliminate choices A, B and D because the electrophoretic patterns associated with the diseases mentioned in those answer choices do NOT match the pattern in the unknown lane. This information alone is enough to determine that the patient has Hemoglobin C Disease. Additionally, note that the patient is only producing hemoglobin C. This is what you would expect with hemoglobin C disease.



Question 5

In the United State, the National Collegiate Athletic Association (NCAA) recommends testing athletes for susceptibility to sickle cell. A young athlete who demonstrates no symptoms is tested by means of hemoglobin electrophoresis at pH 8.4, and the following pattern appears. What do you suspect?



- A) Sickle Cell Disease (SS)
- B) Hemoglobin SC Disease (SC)
- C) Normal patient
- D) Sickle Cell Trait (AS)

Answer 5

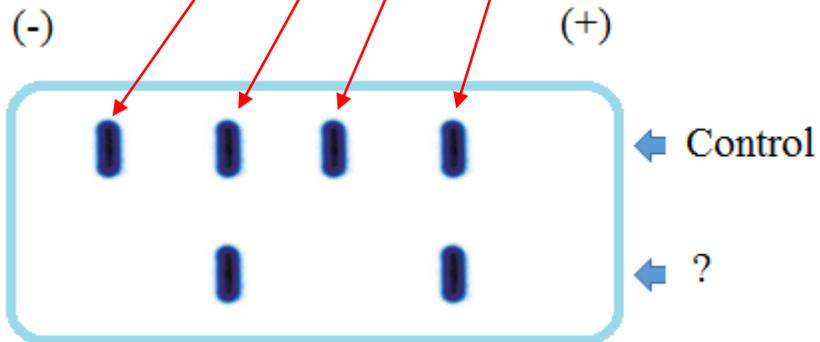
D) Sickle Cell Trait (AS)

We know that this electrophoresis was performed at pH 8.4. Do you remember the diagram I asked you to memorize? Did you draw the version used at pH 8.4?

- CSFA +
pH 8.4

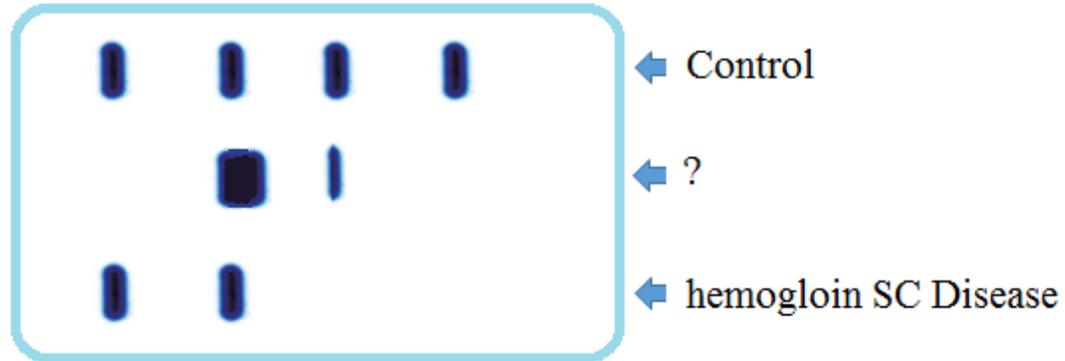
+CSAF-
pH 6.2

This is what electrophoresis looks like on the medium that is used at pH 8.4. Based on the control lane, you know where C,S,F,A should appear on this medium. This patient clearly demonstrates production of Hgb A and Hgb S. Thus, this patient has sickle cell trait (AS).



Question 6

A 16 year old comes into the ER with intense pain. She describes her pain as very sharp and very intense. She appears very pale and hemoglobin testing confirms a low hemoglobin of only 6.0. Hemoglobin electrophoresis is performed at pH 8.4 and it reveals the following. What do you suspect?



- A) Normal hemoglobin
- B) Hemoglobin SC disease
- C) Sickle Cell Disease
- D) Sickle Cell Trait

Answer 6

C) Sickle Cell Disease

We can rule out answer choice B because the patient's pattern is different from the pattern seen with Hemoglobin SC Disease, which is shown in the third lane. Since the question tells you that electrophoresis was performed at pH 8.4, draw out the pH 8.4 hemoglobin electrophoresis pattern that you should have memorized by now. **The location of the bands in the third lane clues us in on the location of the anode and cathode. Recall the pattern! Remember that at pH 8.4, the cathode is closest to C & S.** So now that we have determined the position of cathode (-), the anode (+) and C and S, we are able to determine the positions of Hgb F and Hgb A. This patient demonstrates production of mostly Hgb S and a small amount of Hgb F. What does that describe? Coupled with the symptoms, we can be absolutely certain that the patient's pattern points to Sickle Cell Disease.

