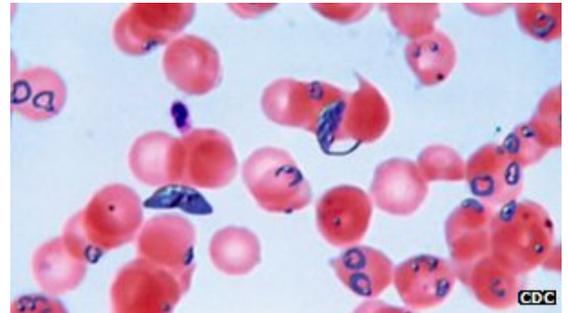


The Parasite Predicament

Diagnosing and Investigating the Transmission of an Infectious Disease

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A 36 year old woman has been rushed to the hospital. Her symptoms are similar to those of the flu, but she has not responded to antiviral influenza medicine. The patient's family history is recorded, as well as a recent travel record. The travel record from the last two years includes a camping trip in the northwestern United States, and a cruise around the Mediterranean.

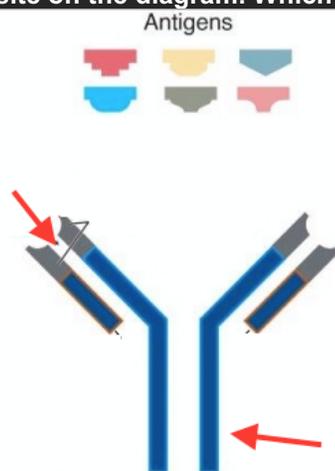


The patient's family history reveals that the patient's maternal aunt and paternal grandfather had Sickle Cell Disease, a heritable disease. The patient is being tested for Sickle Cell Disease, but these results have not been received yet. Sickle Cell trait (the state of being a carrier for sickle disease) may reduce the severity of malaria because it changes the shape of the red blood cell where the malaria parasite lives. The malaria parasite does not fit into a sickle shaped red blood cell.

Since the patient does not know if she is a carrier for Sickle Cell trait, and based on the patient's recent travel, the patient will be tested for malaria. Malaria is a parasite-borne infectious disease. The mosquito's bite introduces the parasite from the mosquito's saliva into the person's blood. The parasite then travels to the liver where it matures and reproduces. The parasite ruptures from the liver and infects red blood cells and multiplies further. This cycle causes fevers, headache, shivering, joint pain, vomiting, yellowing of the skin (jaundice), retinal damage and convulsions. A hallmark of malaria is the cyclical occurrence of sudden coldness followed by shivering, fever and sweating every 36-48 hours.

To test your patient for malaria, you will use an enzyme-linked immunosorbent assay (ELISA), which will demonstrate the presence of malarial antibodies with color change. An ELISA tests for the presence of a specific antigen for a particular antibody. Antigens serve as the target for the receptors of an immune response. Antibodies are large Y-shaped proteins that identify and neutralize pathogens.

Label the antibody and receptor site on the diagram. Which antigen is going to bind to the antibody?



MATERIALS

Positive control
Negative control
Patient's sample

Primary Antibody
Secondary antibody
Enzyme substrate

12-well coated microplate strip
Micropipettes and tips
Wash buffer (saline and Tween())

PART I - MICROPLATE PREPARATION

- 1. Label the first three wells of your microplate strip with "+" for the positive control, the next three wells with a "-" for the negative control and the next three wells with "p", for your patient's sample

Sample	Tube Label	Well numbers
Positive control		
Negative control		
Patient's sample		

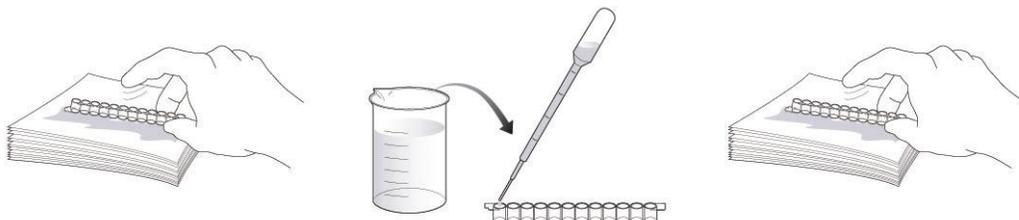


- 2. Transfer 50 μ L of positive control into the designated wells.
- 3. With a new micropipette tip, transfer 50 μ L of negative control into the designated wells.
- 4. With a new micropipette tip, transfer 50 μ L of your patient's sample into the designated wells.
- 5. Wait 5 minutes to allow the proteins to bind to wells.

QUICK CHECK: What does the positive control contain that the negative control does not?

WASHING WITH BUFFER

- 6. Tip the microplate strip upside down on the provided stack of paper towels. Gently tap.
- 7. Using the 100-1000 μ L micropipette, add 350 μ L of wash buffer to each well. Be careful not to spill into neighboring wells.
- 8. Empty the contents onto paper towels. Tap gently (see figure below). Remove top paper towel. **Repeat steps 6-8.**



ANTIBODY ADDITION

- 9. Use a new micropipette tip to add 50 μ L of Primary Antibody (PA) into all wells.
- 10. Wait 5 minutes for the primary antibodies to bind to their targets

QUICK CHECK: What is the antigen-antibody relationship?

- 11. Wash the wells (step 6-8) **twice**.
- 12. Use a new micropipette tip to transfer 50 μ L of secondary antibody (SA) into all wells.
- 13. Wait 5 minutes for the secondary antibodies to bind to their targets.

QUICK CHECK: What is the importance of washing the wells (specifically with Tween)?

QUICK CHECK: Why is it important to wash the wells in between the addition of the primary and secondary antibodies?

- 14. Wash the wells (steps 6-8) **three times**.
- 15. With a new micropipette tip, add 50 μ L of enzyme substrate (SUB) to each well.
- 16. Wait 5 minutes. Positive samples will begin to turn blue.

PART II – RESULTS AND ANALYSIS

Does your patient test positive for the malaria parasite?

If yes, what conclusions can you make about the patient's pending test results for Sickle Cell trait? If no, what next step recommendations would you make?