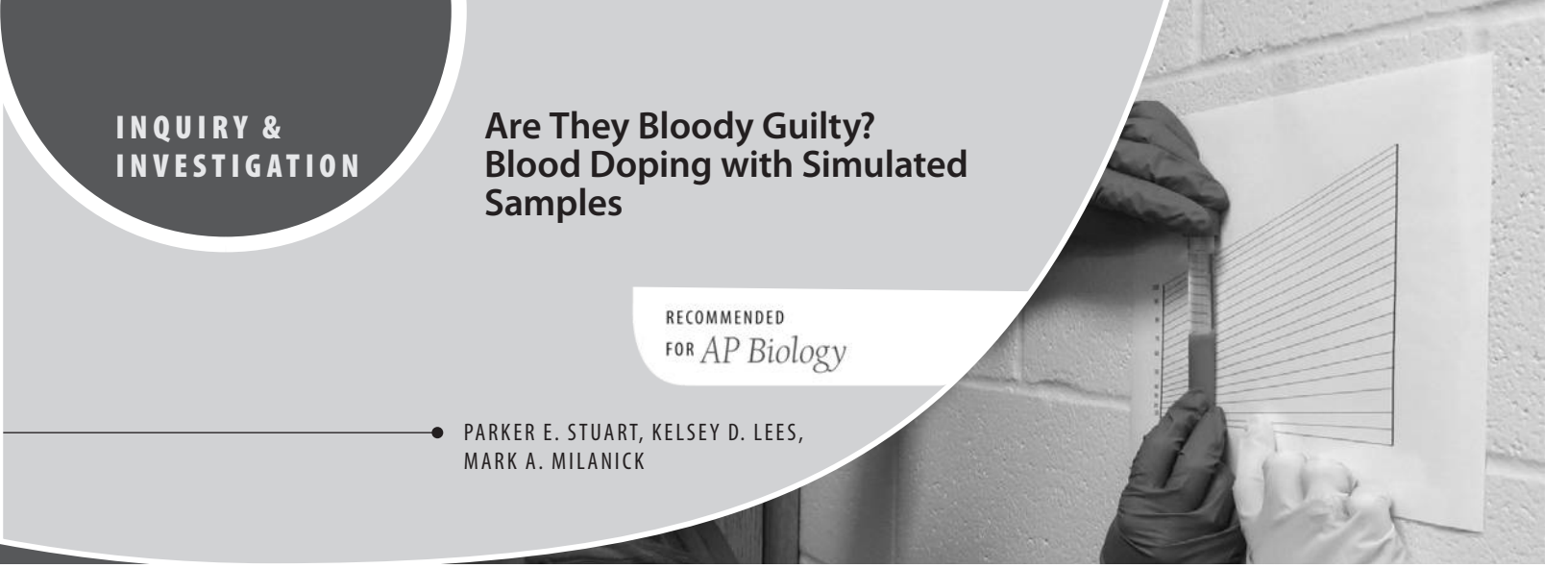
INQUIRY & INVESTIGATION

Are They Bloody Guilty? Blood Doping with Simulated Samples

RECOMMENDED FOR *AP Biology*



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Abstract

In this practice-based lab, students are provided with four Olympic athlete profiles and simulated blood and urine samples to test for illegal substances and blood-doping practices. Throughout the course of the lab, students design and conduct a testing procedure and use their results to determine which athletes won their medals fairly. All of the materials, which simulate the blood, urine, and testing compounds, are available at the grocery store. This real-world problem engages students to think about blood doping, hormones associated with red-blood-cell production, and detection techniques employed by the World Anti-Doping Agency. The Olympics, as well as the news coverage of Lance Armstrong's admission to blood doping in 2013, makes this lab more relevant to students' lives, which is supported by our students' reactions to the lab.

Key Words: *Hematocrit; erythropoietin; inquiry; experimental design; scientific practices.*

It can be challenging for students to apply material learned in Anatomy and Physiology to real-world situations. To help rectify this problem, the *Next Generation Science Standards* (*NGSS*; NGSS Lead States, 2013) focus on the practices of science, with the hope that exposing students to what scientists do will help clarify the relevance of science to everyday life. Immersing students in real-world problems and having them apply their knowledge to these problems also increases student engagement (Dinan, 2005). For example, a student may learn the function of erythropoietin (EPO), yet fail to connect it to hematocrit levels in cases of athletes who blood-dope.

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Similarly, students learn laboratory techniques but fail to understand how these techniques are used outside of the teaching laboratory. One such technique, used specifically in this lab, is simulated fluorescence (sometimes called "photoluminescence"). This technique is used to detect and quantify small amounts of molecules such as proteins and hormones. Fluorescence is the basis for the forensic use of fluorescin to detect blood at a crime scene and is used by the World Anti-Doping Agency to detect blood doping in athletics.

This practice-driven laboratory activity is the collaborative product of a science educator, a high school science teacher, and a scientist. We sought to transform a step-by-step physiology lab into one focused on scientific practices to meet some of the *NGSS* performance standards (see Table 1), which include planning and conducting investigations collaboratively to produce data as evidence, and showing relationships among variables (NGSS Lead States, 2013). By the conclusion of the lab, students will understand how elevated levels of hormones, plasma proteins, and red blood cells can indicate diseases or blood-doping practices. The use of blood and urine in classrooms can be difficult because of the potential-pathogen precautions required in case of a spill. To avoid these difficulties, materials readily available from the grocery store are used to simulate the blood and urine samples.

The laboratory exercise is designed to be done in two 50-minute class periods, although it could be extended, depending on student ability and class length. The first session introduces the scenario as the students become familiar with concepts and detection techniques through guiding questions. Students also design a procedure to analyze the athletes' blood and urine samples. In the second session, students carry out the procedures to collect evidence and share their results with the class to collaboratively assess whether the athletes deserve their medals. The exercise culminates with an assignment in which individual students prepare a letter, based on the evidence collected for each athlete, in response to the Olympic Committee's medal decisions.

○ Scenario

This fictitious case involves the Olympic Medal Committee investigating four athletes who recently won medals. The committee

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requires testing medal winners' samples. If the athletes are found to have used illegal substances, their medals could be stripped. Each of the four athletes has submitted a vial of blood and a urine sample for testing. This scenario challenges the students to learn, or review, components of blood, the relationship between hematocrit levels and associated diseases, blood-doping practices, and detection techniques using labeled antibodies to test each of the vials. Students will collaborate and use their results to confirm whether the athletes' samples adhered to Olympic rules or if their medals should be revoked.

At the start of the activity, a letter from the Olympic Committee is read to the class to set the stage for the scenario. The letter is personalized by the teacher and stresses the importance of accurate results since the athletes' careers are resting on the results. Next, students are given the four athlete profiles and a set of guiding questions and are separated into groups of three or four students. A concept map pertaining to blood doping is collaboratively created by the class to assess their prior knowledge (see Figure 1). Each group is assigned an athlete for testing on the following day. At the end of the first day, students should have answered all of the guiding questions using web resources, class notes, or their textbooks.

On the second day of the activity, students are introduced to the techniques they will use to gather evidence about their athlete, including the athlete's hematocrit and urine EPO levels. For the hematocrit test, four vials of blood (red food coloring, soy milk, and vegetable oil) simulate blood that has been centrifuged. Four urine samples (yellow food coloring mixed with either water or laundry detergent) represent a fluorescent test to detect each athlete's EPO level. After all the evidence is gathered, the students share their results with the class for use in their individual letters. For guiding questions, athletes' profiles, hematocrit scale, and class letter, see http://dalton.missouri.edu/investigators/milanickm.php.

Table 1. *NGSS* performance standards met by the lab activity.

Science and Engineering Practices	
Developing and using models	Develop and use a model based on evidence to illustrate the relationships between systems or between components of a system. (HS-LS1-2)
Planning and carrying out investigations	Plan and conduct an investigation individually and collaboratively to produce data to serve as the basis for evidence. (HS-LS1-3)
Disciplinary Core Idea	
Structure and function	Systems of specialized cells within organisms help them perform the essential functions of life. (HS-LS1-1)
Structure and function	Feedback mechanisms maintain a living system's internal conditions within certain limits and mediate behaviors. (HS-LS1-3)
Connections to Nature of Science	
Scientific investigations use a variety of methods	Scientific inquiry is characterized by a common set of values that include logical thinking, precision, open-mindedness, objectivity, skepticism, replicability of results, and honest and ethical reporting of findings. (HS-LS1-3)

○ Hematocrit, Erythropoietin, & Blood Doping

In this activity, students are tasked to review and discuss the cellular components of blood and how this relates to hematocrit and blood doping. Blood is made up of erythrocytes (red blood cells), leukocytes (white blood cells), platelets, and plasma. When the blood is in suspension, all of the cells mix together and appear as the familiar red fluid. When centrifuged, the cells separate according to density. Red blood cells settle at the bottom of the test tube, white blood cells settle above the red blood cells, and plasma resides at the top. The percentage of blood that consists of red blood cells is known as an individual's "hematocrit level." Typically, this is

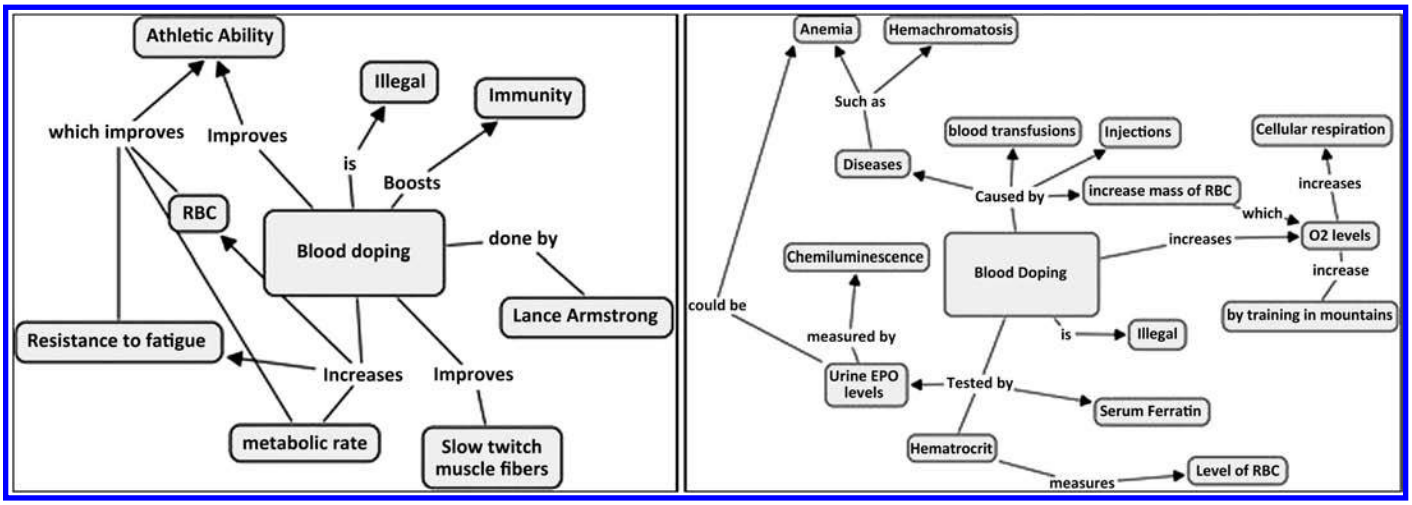

Figure 1. Comparison of student-generated concept maps, (left) prelab and (right) postlab.

about 40% of the blood in adults (females, 33–40%; males, 38–45%). Abnormal hematocrit levels can be caused by certain diseases, as well as by pharmaceutical drugs.

One possible cause of low hematocrit is anemia, commonly caused by a deficiency in iron, vitamin B12, or folate. All of these are important for red-blood-cell synthesis. If the anemia is a result of nutritional deficiency, it can be treated by increasing the intake of the appropriate nutrient (iron, B12, or folate). Another cause of anemia is a reduction in the production of EPO. This can occur in patients with renal disease, because the kidney synthesizes EPO. In these cases, the medical treatment for anemia often includes injection of synthetic EPO.

Blood doping can be a cause of elevated hematocrit unrelated to a disease state. Doping can be done through several means. One option is transfusing packed red cells into the body prior to competition. Anallogeneic transfusion places compatible red blood cells from a donor into the athlete. This method of blood doping has the drawback of requiring compatible blood, since incompatible blood could cause agglutination inside the athlete's body. If this occurs, the vessel become obstructed and causes extreme pain or even death. Until recently, detecting allogeneic transfusion was difficult. However, fluorescent activated cell sorting can detect minor mismatches of antigens and Rh factors between donor and recipient (Arndt & Kumpel, 2008). To avoid detection, athletes have begun using autologous transfusions, which differ from allogeneic transfusions because the athlete transfuses his own blood back into himself. This method avoids the compatibility problem and is undetectable because all antigens match.

A second form of blood doping involves injection of synthetic EPO. The synthetic EPO stimulates the red bone marrow to produce more red blood cells, resulting in excess production of red blood cells in healthy athletes. The reason athletes want elevated red-blood-cell levels lies in their function. Red blood cells carry oxygen, and as more red blood cells are produced, more oxygen is available to tissues. In endurance sports, such as cycling and long-distance running, oxygen demand is high and the excess red blood cells allow the athlete to fatigue more slowly, resulting in increased performance. Normal levels of EPO are 10 mU/mL. However, under low-oxygen stress or if pharmaceutical EPO is being used, EPO levels may increase to 10,000 mU/mL. Normally, EPO is filtered by the kidneys, with small amounts excreted through the urine.

One legal form of blood "doping" is training at high elevations, where atmospheric oxygen levels are lower. When the body's blood oxygen levels become low as a result of higher-elevation training, the body's natural response is to produce more EPO. A side effect from the increased red blood cells is higher hematocrit levels resulting in a condition known as "secondary polycythemia." Eventually, the body acclimates to the elevation and red-blood-cell production remains high. Upon returning to lower elevations, the body slowly decreases red-blood-cell production because more oxygen is available. The idea behind this training technique is that it is a natural way to get the same effect as synthetic EPO use. Results from this type of training are inconclusive, with some athletes who live at high elevations and train at lower elevations outperforming the high-elevation trainers (Levine & Stray-Gundersen, 2001).

How to Test the Samples

One way to measure hematocrit is by moving the centrifuged blood horizontally across a hematocrit scale until the top of the plasma reaches the 100% line. To begin, place the centrifuged blood sample so that the bottom of the test tube resides on the x-axis. The top of the plasma (vegetable oil) will be off the scale. Move the tube horizontally until the plasma level aligns with the 100 diagonal line on the hematocrit scale. The packed red blood cells (red soy milk) will transect a second line associated with the sample's hematocrit level (see Figure 2).

To test EPO levels, a concentrated drop of urine is mixed with labeled antibodies that bind to EPO. The antibodies are labeled with a fluorescent dye. In a sandwich assay, a capture antibody is added that binds to another place on EPO and allows one to separate the EPO-labeled antibody complex from the labeled antibody. To test urine EPO levels, a drop of the concentrated urine antibody complex is placed on filter paper and exposed to black light.

Materials

All the materials for creating the samples are available at the grocery store.

- Plain soy milk
- Vegetable oil
- Colorless laundry detergent (we use All brand "Free and Clear")
- Eight 12 × 75 mm test tubes
- Test tube rack
- 410-nm black light (we use a black-light flashlight in our class from Amazon.com)
- UV-blocking safety goggles
- White coffee filters
- 1-mL disposable pipettes
- Red and yellow food coloring
- Distilled water

Figure 2. Students measuring hematocrit levels.

Table 2. Contents of the sample tubes.

Test	Athlete 1	Athlete 2	Athlete 3	Athlete 4
Hematocrit Level	*4 mL Red Soy Milk (~40% hematocrit)*, vegetable oil	*6 mL Red Soy Milk, (~55% hematocrit)*, vegetable oil	*5 mL Red Soy Milk, (~50% hematocrit)*, vegetable oil	*3 mL Red Soy Milk, (~30% hematocrit)*, vegetable oil
EPO	Water, yellow food coloring, *0.2 mL detergent*	Yellow food coloring, *2 mL detergent*	Water, yellow food coloring, *0.2 mL detergent*	Yellow food coloring, *2 mL detergent*

Note: Italics indicate differences between samples.

Table 3. Expected student lab results, with disease states in parentheses.

Test	Athlete 1 (Clean)	Athlete 2 (Blood doping with EPO)	Athlete 3 (Secondary polycythemia)	Athlete 4 (Anemia)
Hematocrit Level	Normal	High	High	Low
EPO	Normal	High	Normal	High

○ Creating the Preparation Room & Samples

To test the athletes' EPO level, set up a dim or dark room to simulate a laboratory prep room for fluorescence. The room does not need to be pitch black but should be dark enough that the students can see if their samples fluoresce. An easy way to test whether the prep room is dark enough is to test the athletes' samples as you create them. If the fluorescence is hard to see, make the room dimmer and/or make the sample stronger.

To create the hematocrit blood samples, plain soy milk and red food coloring are mixed in a stock solution until a blood-like red color is obtained. Soy milk is used to mimic the light-scattering property of human blood. Next, four test tubes are filled using pipettes with varying amounts of the simulated red blood cells. One tube is filled with a normal hematocrit level, two are filled with abnormally high levels, and one is filled with an abnormally low level. Make sure to consult the hematocrit scale to fill the tubes to their respective volumes. Finally, vegetable oil is added to the tubes to bring the total volume to 12 mL (100% line on the hematocrit scale). Repeat the process until all the tubes are filled with the corresponding contents (see Table 2).

The athletes' urine EPO levels are created by a mixture of yellow food coloring, laundry detergent, and water. Normal levels of EPO are a mixture of water, detergent, and yellow food coloring, whereas high EPO levels are yellow food coloring and laundry detergent (see Table 2). A total volume of 2 mL is used for the EPO samples. Normal urine does contain small amounts of EPO, so 0.2 mL of laundry detergent is added by pipette to the "normal" EPO test tube and is slowly inverted until the detergent is mixed. Remember that vigorous mixing will cause the detergent to form bubbles – so be gentle! Place a drop of simulated urine onto the coffee filters and shine with the black light to test the fluorescence. Precautions must be taken with the black light because wavelengths below 410 nm may cause damage to the corneas and, possibly, damage to the retinas as well. Students should wear UV protective eye wear and a lab coat when using the flashlights. A standard EPO tube is created and placed in the prep room for student comparison. Table 3 shows the expected results of the four athletes' tests.

The preparation time for the activity may be a concern to a busy teacher. We took 30 minutes to create all the samples, but a single teacher may take longer, especially if you have a large class. Both the hematocrit and EPO samples can be created well in advance of the activity. The hematocrit samples can be stored for up to a month in a refrigerator, whereas the EPO samples can be stored at room temperature indefinitely.

○ Student Reactions & Conclusions

Our high school students were excited to learn about blood doping because of Lance Armstrong's admission of blood doping in early 2013. The athletes' profiles gave a personal touch and engaged student athletes, who were the same age as the fictitious Olympians. The students were also drawn into the puzzle aspect of the activity because they were personally charged with figuring out whether the medals were won fairly. Furthermore, the hands-on portions of the lab allowed the students to practice and experience how scientists test for blood doping.

Some of the higher-achieving students mentioned that the activity could be made more challenging if we did not guide them as much with the testing protocol. This was contradicted by a second group of students, who said that they could not have developed the protocol from scratch. Depending on their students' ability level, teachers can modify the activity to be more challenging with the addition of a ferritin test (for a description of this more challenging activity, see http://dalton.missouri.edu/investigators/milanickm.php) or by having each group of students test all four athletes, which would increase the preparation time considerably. Since teachers understand the capabilities of their students, we suggest they use whichever form of the activity they feel their students will benefit the most from.

This laboratory activity has several advantages for the classroom. First, the activity reflects the vision of the *NGSS* in its focus on scientific practices. The activity also engages students with solving a case using evidence they gathered, which is another goal of the new standards. Third, the content is relevant to students' lives because of the publicity surrounding Armstrong's blood doping, which reinforces the real-world application of ideas learned in the classroom. Finally, the use of materials from the grocery store to simulate blood and urine gives teachers a safe alternative for the classroom.

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