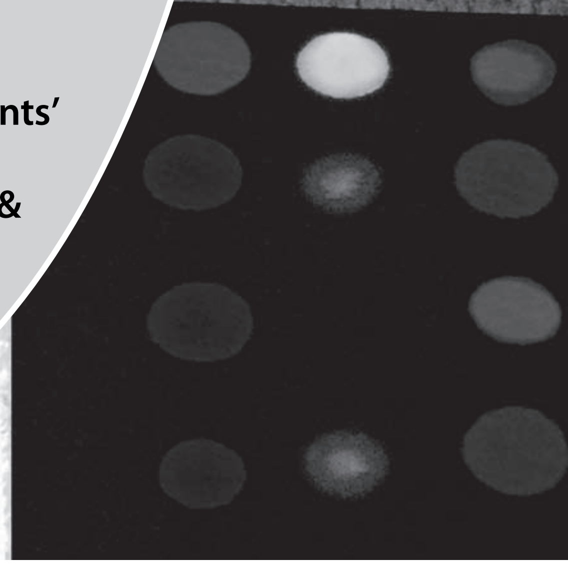

INQUIRY & INVESTIGATION

Did Foul Play Lead to the Patients' Comas? Investigating Possible Attempted Murders by Insulin & Related Compounds

KATHERINE MEIDL, SAMANTHA BROWNING, MARGARET URSCHLER, BASIMA KHAN, MARK MILANICK

Abstract

The action of hormones such as insulin in contributing to life-threatening diseases such as diabetes may be difficult for students to understand. To teach students the critical details of the regulation of blood glucose and the different types of diabetes, we created a laboratory exercise using a five-patient hypoglycemic–hyperglycemic coma case.

Key Words: *Insulin; glucose; C-peptide; diabetes; diabetes mellitus.*

Police Officer: *Seems mighty suspicious to have five patients in a coma because of extremely abnormal glucose levels.*
Health Care Provider: *You are right, it is unusual. Do you have any reason to suspect foul play?*
Police Officer: *Only that each relative that found the patient in the coma would have a motive.*
Health Care Provider: *Well, I can order some specialized blood tests that would tell you whether foul play was involved or not.*
Police Officer: *How would the blood tests help?*

How hormones like insulin can contribute to life-threatening diseases like diabetes can be a hard concept for students to grasp. To teach students the critical details of the regulation of blood glucose and the different types of diabetes, we created a laboratory exercise using a five-patient hypoglycemic–hyperglycemic coma case.

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○ The Scenario

In this laboratory exercise, students play the role of health care providers and are presented an assignment from the hospital in which they work. The hospital had five patients admitted during the night, and they were all in comas. Some members of each family believe that foul play was involved. The patients all had blood drawn and sent to the lab to find the causes behind the comas. After examining the samples, the students will use their physiology knowledge to determine which sample goes with each diagnosis. The students

- measure blood sugar levels using urine glucose test strips. Both very high and very low blood sugar levels can cause a coma. A person who has low blood sugar levels should be administered glucose, but clearly a person with high blood glucose levels should not be given more glucose.
- measure simulated insulin levels. These levels will be high if too much insulin is injected and will be low if a person with type 1 diabetes was injected with saline instead of insulin.
- measure simulated C-peptide levels. These levels will be high if the patient has an insulinoma or takes a sulfonylurea, as these both will release insulin and C-peptide from the pancreas.
- measure simulated sulfonylurea concentrations. These levels will be high if the patient has taken sulfonylureas.
- examine a blot to determine the amount of fluorescence, which is proportional to the amount of compound, for the three simulated samples of insulin, C-peptide, and sulfonylurea. (For classes that are not able to make a blot, the students can be provided either Figure 2 or the values of the hormones as given in Table 3.)

Different measurements of the samples correspond to specific physiological conditions. For example, a person diagnosed to be in a hypoglycemic coma due to being injected with too much insulin would have high insulin levels, low C-peptide levels, low glucose levels, and no sulfonylurea drugs (see below) present in his blood (see Figure 1). Each team will test for insulin, C-peptide, and sulfonylurea drugs using simulated fluoroimmunoassays. Glucose levels will also be tested using glucose test strips. For each sample, the students must fill out a lab report explaining the findings and

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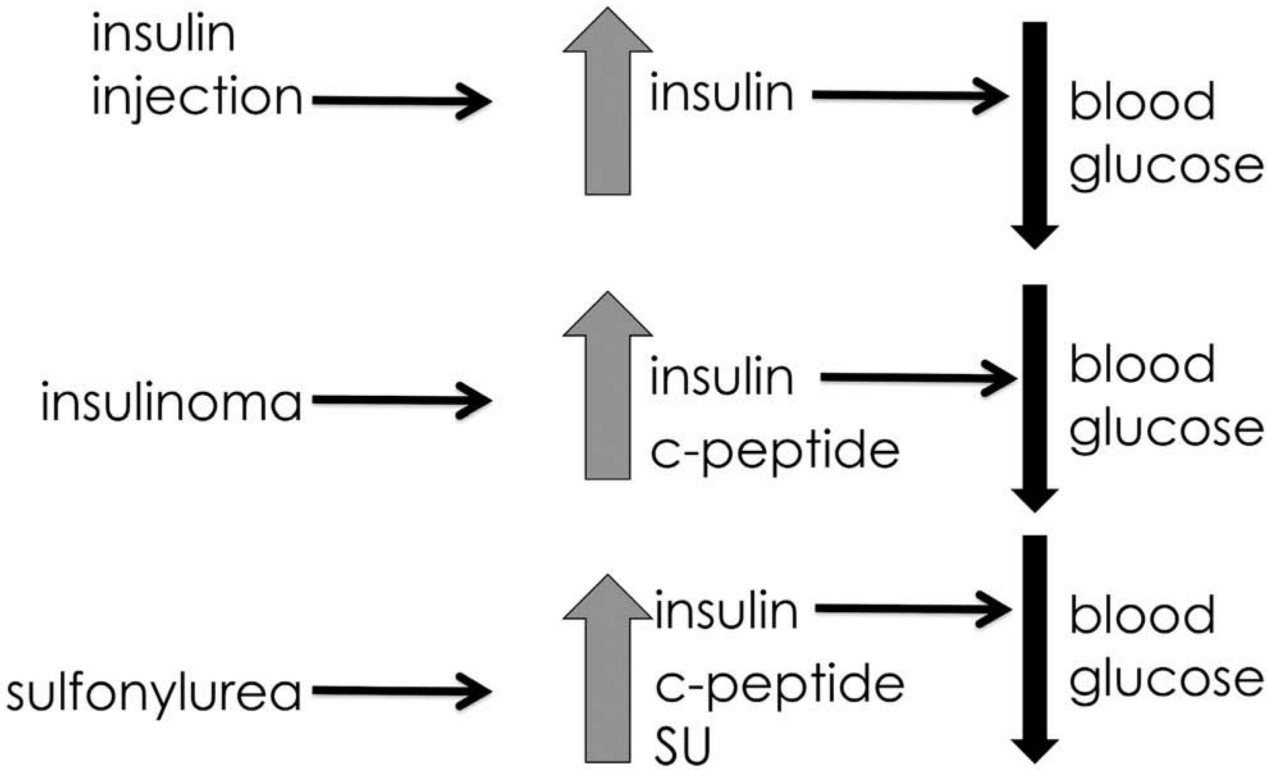

Figure 1. Insulin will lower blood sugar. The insulin could be present as a result of an insulin injection, an insulinoma (a tumor that secretes insulin), or taking a sulfonylurea drug (SU). An insulin injection would not contain C-peptide; the only time SU will be found in the blood is if it has been taken or administered.

how these results led each team to their conclusions about matching the samples. By looking at the lab tests, students will be able to actively apply what they learned in class about the physiology of glucose control and see how various hormones in the body affect a person in different ways. In addition, they will learn how imbalances can cause life-threatening illnesses like diabetes and debilitating states like comas.

○ Patient Histories

Susan: The female patient (46 years old) was carried into the emergency room (E.R.) around 2 a.m. The patient was unresponsive. The son of the patient says he found his mother passed out on the bathroom floor after a New Year's Eve party, which she had left early because she was light-headed. The son says she had a few drinks at the party, but nothing to worry him. However, the husband of the patient says she had quite a few drinks and that he believes she just had too many. At a later time, the son asks the doctor for a blood test to be run. He believes his mother's husband may have poisoned her, because his mother told him earlier in the night she would be divorcing him. The patient does not have a history of diabetes.

Ivan: An elderly man (67 years old) is living at home with several illnesses, including Alzheimer's disease, arthritis, and type 1 diabetes. He has hired a home health aide to help him throughout the day, because he cannot live independently and has a large home. His aide brought him to the E.R. in the morning because she could not wake him. His nurse left the house at the end of her shift, leaving her patient in the care of his grandson overnight. The grandson is living with his grandfather, in exchange for helping with his grandfather's care. When the nurse came back in the morning, she could not rouse her patient. The doctor asks what medicines the patient has taken recently, and the aide responds, "Just an insulin injection with dinner around five o'clock the night before."

Candi: The patient (15 years old) was brought into the E.R. in a hyperglycemic coma this evening by her mother. Her mother says the patient was at a cousin's birthday party this afternoon. Her daughter, Candi, is a type 1 diabetic and took her insulin injection before the party, knowing there would be a lot of cake, ice cream, and other sugary foods. The patient was supposed to spend the night at the cousin's slumber party later that evening. However, her mother had to take the patient home after a few hours because she reported feeling light-headed. Upon returning home, the patient had to lie down to rest. Later, the mother could not rouse the patient. The doctor suspects there is an issue with the insulin that Candi is injecting, because the daughter has been in the E.R. a few times this year with the same problem.

Olga: The patient (22 years old) was brought into the E.R. by a roommate. The roommate found the patient passed out in the shower this morning. The roommate says that the patient was not drinking the night before. In fact, the patient has been feeling light-headed and dizzy all week, so she stayed home to rest last night. When the mother arrives, she says the patient is rarely sick, and something very unusual must have occurred. She does not trust her daughter's roommates.

Isaac: The patient (25 years old) was brought into the E.R. by his wife, who was not able to wake him this morning. She had been gone on business but knows that he went out bowling with friends last night. He has type 1 diabetes. She brought in his medicine bag, which had the insulin-injection supplies showing that he injected insulin last night; however, his insulin serum does not look as low as it should be if he had two injections that day. She also noticed that his bag is much messier than it should be. She believes someone else may have altered her husband's medicines. She suspects the high school chemistry teacher, who is competing for the job of principal with her husband and who knows he has diabetes.

○ Murder by Insulin & Related Compounds

It is important for the body to have the right amount of blood sugar (glucose). Normal glucose levels in the fasting state are 70–100 mg/dL (3.9–5.6 mM). Sugar is used as an energy source for cells; however, many cells, including skeletal and cardiac muscle, can also use fatty acids. By contrast, glucose is the almost exclusive energy source for the brain. If the brain does not receive the right amount of glucose, it will not be able to function properly and the person can die. As an analogy for the body's unique fueling system, consider a modern hybrid car; the engine of the car can run using electricity or gasoline, but the car's computer (brain) exclusively uses electricity.

While very low blood sugar levels (hypoglycemia) can be fatal, it is also true that very high blood sugar levels (hyperglycemia) can also be fatal, just as too much electricity could ruin the car's computer. Hypoglycemia is fatal because both nerve cells and muscle cells need to function. Without enough energy, the nerve cells in the brain cannot signal the respiratory muscles to contract and the respiratory muscles do not have enough energy to contract. With too much blood sugar, one problem that occurs is that the body and its cells lose too much water. This makes the cells malfunction.

In order to regulate blood sugar levels, the body uses the hormones insulin and glucagon, as well as noradrenaline and cortisol; for this laboratory exercise, we stress only insulin, whose role is to lower blood sugar levels. Glucagon, noradrenaline, and cortisol all tend to increase blood sugar levels.

After you eat a meal high in sugar, your blood sugar increases. This increases the sugar content of your pancreatic beta cells. The increase in pancreatic beta-cell sugar causes insulin to be released from the pancreas. Insulin binds to receptors on skeletal muscle cells, liver cells, and fat cells. This leads to an increase in the amount of blood sugar that ends up inside the cells; as more glucose ends up in the cells, there is less glucose in the blood, so the blood glucose levels fall. When there is too little blood glucose, the insulin levels go down, and glucose is not removed from the blood. This helps keep enough glucose in the blood to fuel the brain.

Diabetes mellitus is a group of diseases in which the body has too much glucose in the blood, and it is not being properly absorbed and utilized by the body. In type 2 diabetes, the initial problem seems to be that the insulin receptors do not respond as well to insulin. As an analogy, consider you are talking to your grandparent: your voice is analogous to insulin, in that both are signals. Your grandparent's ears are analogous to the insulin receptor. When your voice hits their ears, they respond. In type 2 diabetes, your grandparent's ears do not hear as well, so they do not respond when you talk in your normal voice. However, if you raise your voice (have more insulin), they do respond. Therefore, people with type 2 diabetes are often given drugs that cause their pancreas to release more insulin – a bit like raising your voice.

Pancreatic beta-cell insulin release can be increased by a class of drugs called *sulfonylurea drugs*, or "SU" for short (Thulé, 2012). These drugs cause the body to produce its own insulin and increase the pancreatic beta-cell insulin release. They are useful for treating patients with type 2 diabetes.

With type 1 diabetes, the problem is primarily due to the fact that the body's pancreatic beta cells are being destroyed by the immune system, so the body cannot make enough insulin. People with type 1 diabetes give themselves insulin shots to control their blood sugar. To continue our child/grandparent analogy, this would be like the child having laryngitis. Telling the child to speak louder is not going to help, because the child has no voice. But another child can speak and warn the grandparent about some danger. The other child is taking the place of synthetic insulin. An overdose of insulin or SU can lead to such low blood sugar that the patient can go into a coma or even die. To determine whether SU was given, a health care worker can order a test that measures whether there is SU in the blood (SU is detected only in someone who has taken an SU).

If someone were given an overdose of insulin, they would have very high insulin levels. However, there is another noncriminal explanation for very high insulin levels; the patient could have an insulinoma, a tumor that can secrete insulin even when blood sugar drops too low. To understand the blood test that can be used to distinguish a patient with an insulinoma from a patient who has had too much insulin injected, one needs to understand how the pancreatic beta cell makes insulin.

Insulin is made up of amino acids that form a chain, sort of like beads on a necklace (Brandenburg, 2008; Stansfield, 2012). A chain like this is called a *peptide* if it is short, or a *protein* if it is long. Insulin consists of two peptides, A and B, linked together (or cross-linked). Proinsulin is a single-chain peptide that is processed to form insulin. The proinsulin contains the A and the B peptides, as well as a linking peptide in between, called the *C-peptide*. The insulin molecule folds in a way that allows the A and B ends to meet and cross-link. After the cross-links form, the C-peptide piece is cut out, leaving insulin as the cross-linked A and B peptides. The beta cells in our pancreas release both the insulin and the C-peptide. They are released in a 1:1 ratio, but insulin is broken down faster than C-peptide – so, usually, if you measure the ratio in blood it is 1:3, meaning three times more C-peptide than insulin. Drug companies that supply insulin for patients with diabetes purify the insulin, and this removes the C-peptide. This allows the presence of C-peptide in the blood to be a marker for health care professionals to determine whether the patient is producing insulin on his own. If there is just a high concentration of insulin, without the C-peptide, the health care workers (and police) know that this is synthetic, not endogenous, insulin. C-peptide is thus a marker showing that the body produced the insulin present in the blood or that the insulin is synthetic.

A sandwich immunoassay can be used to measure insulin, C-peptide, and SU (Kwong & Teale, 2002). For example, to measure insulin, an antibody complementing one region of insulin is bound to blotting paper. A second antibody complementing a different part of insulin is labeled with a fluorescent dye, then mixed with the sample containing insulin, so that the second antibody can bind to insulin. This sample is then blotted on the paper containing the first antibody. The insulin bound with the second antibody also binds to the first antibody attached to the blotting paper. All of the second antibody that is not bound to insulin is washed away. The amount of fluorescence remaining is a measure of how much insulin was present.

If the patient did happen to be diabetic and his treatment, such as SU or insulin injections, no longer worked, then he would not be able to lower the amount of blood sugar in his body. This would cause abnormally high levels of blood glucose and would dehydrate the cells in his body, including his brain cells. The severe dehydration can lead to shock, coma, or death (Corwell et al., 2014).

○ Materials

- Distilled water
- Small test tubes
- Test tube racks
- Orange highlighter pen (e.g., Sharpie)
- RIT Whitener and Brightener or laundry soap with whitener
- Turmeric (e.g., Spice Island brand)
- Food coloring (e.g., Durkee brand)
- Blotting paper (e.g., Thermo Scientific Pierce Western Blotting Filter Papers 88600)
- Glucose test-strips (e.g., Rapid Response, Diastix, or DiaScreen urine dipsticks)
- Blacklight (e.g., Ecobulb blacklight), desk lab, box with viewing port or ultraviolet flashlights (LEDwholesalers 395 nm UV Ultra Violet 21-LED Blacklight Flashlight, 7305UV395, from Amazon) and goggles (UV Protecting Adjustable Safety Glasses Yellow Tint, 7821, from Amazon).

○ Laboratory Exercise

If we have time, we grab the students' attention for this laboratory exercise by showing a video compilation of insulin murder cases (Table 1). Otherwise, we motivate the students by giving them some actual headlines, journal article titles, or Wikipedia pages that deal with insulin murders (Table 2). We then ask them some questions, for example:

> *How can one tell if someone is given too much insulin in a shot?*
> They will have a high ratio of insulin to C-peptide.
>
> *How is blood sugar controlled?*
> When blood sugar increases, insulin levels rise. The increase in insulin levels allows muscle, fat, and liver cells to remove glucose from the blood. When blood sugar levels fall too low, glucagon, noradrenaline, and cortisol levels increase. Increases in these hormones allow liver cells to release glucose to the blood.

The students are then asked to read background information on glucose and diabetes mellitus. For our large labs (approximately 80–150 students per lab) of sophomore pre-nursing and pre-health-professional students, we find it time effective to provide them with the basic physiology in a seven-page handout to read before the lab, with prelab questions to answer. Other sources include American Diabetes Association (2014) and Conway and Leonard (2014).

When the students arrive in the lab, they work in groups of three to five; we suggest to the students that they will find it helpful to fill out Table 3 as a group in order to help them decide what they expect in the different cases.

To simulate the immunoassays, we pipetted different amounts of fluorescent material onto the blotting paper. The amounts are provided in Table 4, and a typical blot is shown in Figure 2. We have successfully stored these blots for over six months.

There are two different methods for reading the simulated fluoroimmunoassay. In one method, the students use ultraviolet flashlights and ultraviolet safety glasses. In the other method, students use a blacklight and don't need safety glasses. In both cases, it is best to have a dark room or a viewing box. A simple

Table 1. Online videos about insulin murders.

Brief Summary	Source
Real Crime: Angel of Death, a documentary about Colin Norris, a nurse from the United Kingdom, who was convicted of murdering patients in his care by overdosing them with insulin	https://www.youtube.com/watch?v=cqHdXPiXoWc
"Is this nurse serving 30 years for murders that never happened?" by David Rose, *Daily Mail*, May 18, 2013 (newspaper article and accompanying video about Colin Norris)	http://www.dailymail.co.uk/news/article-2326712/Is-nurse-serving-30-years-murders-happened-Compelling-new-evidence-suggests-Angel-Death-innocent.html
"UK nurse accused of killing patients with insulin injection," by PressTV	https://www.youtube.com/watch?v=DNIkeLaHSps
Law & Order season 3, episode 19, "Virus": A computer virus at a diabetes clinic causes the wrong amount of insulin to be given to patients, killing two	http://www.imdb.com/title/tt0629490/

Table 2. Newspaper headlines about insulin murders.

Headline	Newspaper/Source
'Angel of Death' Colin Norris could be cleared of insulin murders	*The Guardian* October 3, 2011
Attempted murder-by-insulin case set for arraignment in Port Angeles this week	*Peninsula Daily News* August 28, 2014
Kenneth Barlow: The first documented case of murder by insulin (1957)	*Journal of the Royal Society of Medicine* 2008, vol. 101, pp. 19–21.
Wallsend aged-care deaths: Nurse faces two charges of murder after deaths of patients	*Sydney Morning Herald* December 17, 2014
Diabetic Brooklyn teen denied insulin after being falsely arrested on attempted murder charges: Suit	*Daily News* August 1, 2014
Insulin was the weapon in murder attempt at Olympic Medical Center, Port Angeles police say	*Peninsula Daily News* June 28, 2014
Two more cases of murder-by-insulin alleged at a nursing home	*McKnight's* March 14, 2014
Arizona woman sent to psychiatric facility in attempted insulin murder	*Las Vegas Review-Journal* December 12, 2013
Insulin poisoning: Murder detectives release male nurse on bail	*Daily Mail* January 8, 2012
Two pictures emerge in insulin murder trial	*Rutland Herald* September 12, 2013
Parkway teachers say insulin given to them instead of flu shot	*KDKA* October 7, 2014

Table 3. This table is given to the students to fill out (with only the information in bold provided). Students are told that the values can be low or high for glucose; low, moderate, or high for C-peptide; low, high, or highest for insulin; and absent or present for SU.

"Problem"	Insulin	C-Peptide	SU	Blood Glucose	Patient
Inject too much insulin	High	Low	Absent	Low	Ivan
No insulin	Low	Low	Absent	High	Candi
Sulfonylurea overdose	High	High	Present	Low	Susan
Insulinoma	High	High	Absent	Low	Olga
Sulfonylurea *and* insulin injection	Highest	Moderate	Present	Low	Isaac

viewing box is a cardboard box with one open end and a tarp to go over the students' heads. A slightly more sophisticated viewing box is a black plastic storage container (18 gallon) holding the desk lamp/blacklight with a viewing hole (2 × 6 inches) cut in the top.

To measure the glucose levels, we made up test tubes containing water or glucose and used urine dipsticks following the directions on the container. The lowest glucose reading on these dipsticks is approximately the normal blood values, so using water guarantees that the dipstick response will mimic hypoglycemia. For the hyperglycemia patient, we used a stock solution of 0.5 g glucose in 50 mL.

After reviewing the results of the blood tests of the patient, each group will determine the cause of the coma and explain the reasoning behind the choice.

We have done this lab with undergraduate students after giving them a 20-minute prelab review of the information, and it takes them 60 to 90 minutes to complete the exercise. It took us about 1 hour to make up the stock solutions and make 12 blots.

Table 4. Solution and blot preparation.

(A) Stock Solutions			
Stock	**Source**	**Preparation**	**Second Step**
Turmeric	Spice Islands turmeric	Make a saturated turmeric solution in rubbing alcohol (91% isopropyl alcohol) by adding 0.5 g in 20 mL of rubbing alcohol	Allow to settle or centrifuge.
RIT	RIT Whitener and Brightener	Make a saturated RIT in rubbing alcohol (91% isopropyl alcohol) by adding 2.5 g in 20 mL of rubbing alcohol	Allow to settle or centrifuge.
Orange highlighter	Sharpie orange highlighter	Remove white bottom piece and take out cylinder, then place in 50 mL tube and add isopropyl alcohol to 50 mL	Allow to sit for 1 hour. Remove solution.
Yellow food color	Durkee yellow food coloring	0.4 mL yellow food coloring + 5 mL water	
Red food color	Durkee red food coloring	0.2 mL red food coloring + 5 mL water.	
Glucose	Glucose	0.5 g glucose in 50 mL of water for a concentration of 1000 mg/dL	

(B) Solutions for Blot				
Tube	**Fluorescent Material**	**Red Food Coloring**	**Yellow Food Coloring**	**Water**
Turmeric high	200 μL turmeric stock		200 μL	
Turmeric low	–		200 μL	200 μL
RIT high	400 μL RIT stock			
RIT med	20 μL RIT stock			380 μL
RIT low				400 μL
Orange high	20 μL orange highlighter	20 μL	100 μL	
Orange low		20 μL	100 μL	

(C) Pipetting the Blot & Creating the Tubes for Glucose Dipstick Testing				
Patient	**SU-Blot Column 1**	**Insulin Blot Column 2**	**C-Peptide Blot Column 3**	**Glucose Tubes**
Susan	Orange high	RIT med	Turmeric high	Water
Ivan	Orange low	RIT med	Turmeric low	Water
Candi	Orange low	RIT low	Turmeric high	Glucose stock
Olga	Orange low	RIT med	Turmeric low	Water
Isaac	Orange high	RIT high	Turmeric high	Water
Pipette 20 μL from the appropriate tube for each point on the blot. The students should be informed that the insulin to C-peptide ratio is normal in Olga.				

○ Protocol Modifications & Additional Inquiries

There is some evidence that C-peptide plays a physiological role (Hills & Brunskill, 2009); in the future, C-peptide might be included with insulin for injections. Students could read about this and debate whether the federal government should require some marker to be added to injected insulin, in order to have a test that replaces the C-peptide levels for determining whether a patient's high insulin was due to injected insulin. This discussion could talk about whether there is any compound that is completely safe that can be added. They should also weigh the relative number of murders (that we know of) due to insulin injection versus the possible harm to patients if even 0.1% has a bad side effect to the marker.

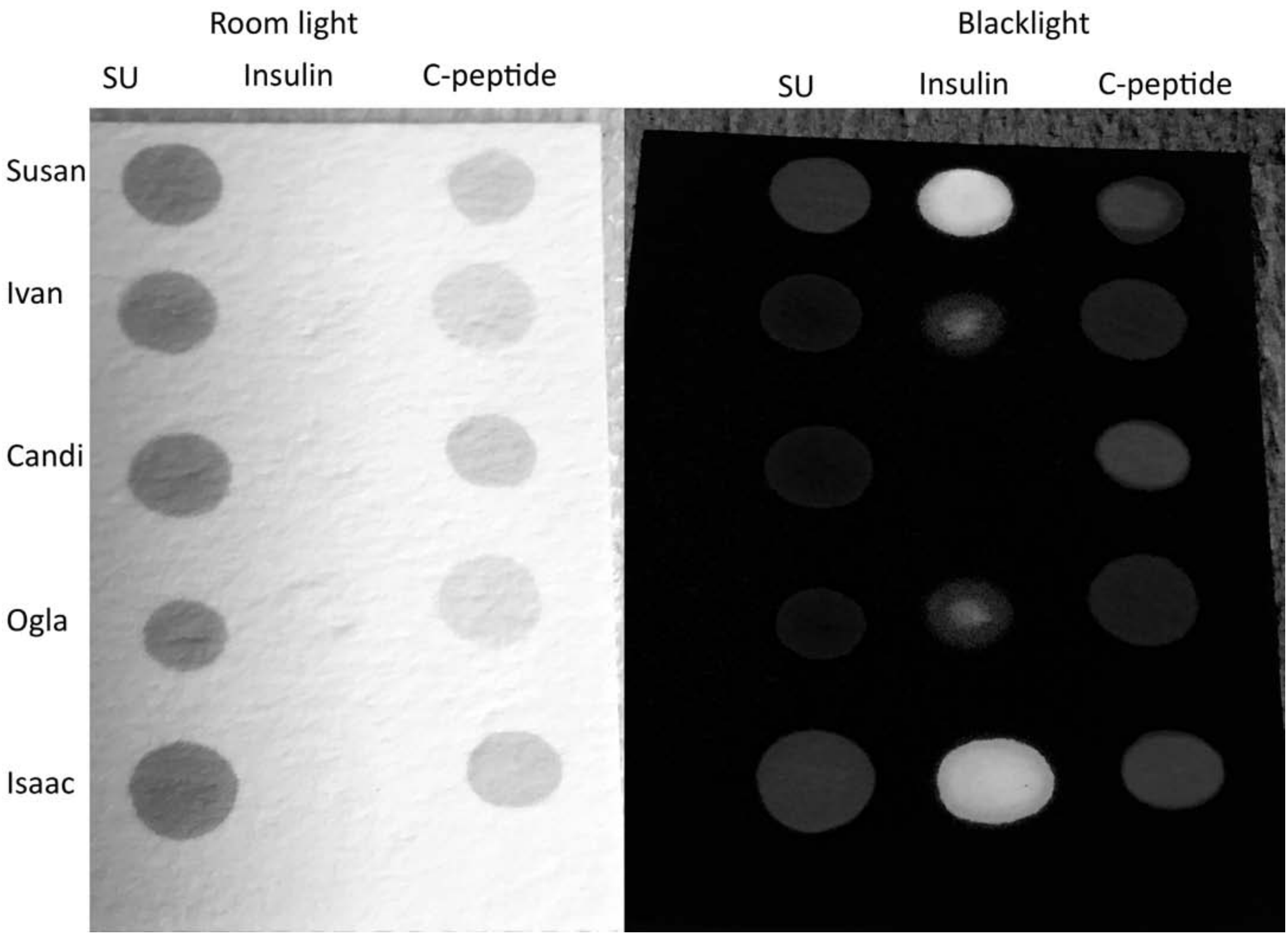

Figure 2. Typical blot for the students to analyze. On the left is the blot in room light; on the right, the fluorescence is visible using a blacklight. The sulfonylurea drug (SU) and C-peptide spots are visible but nearly identical under room light. With the blacklight, one can observe high SU in Candi and Isaac; very high insulin in Susan and Isaac; moderate insulin in Ivan and Ogla; very low or absent insulin in Candi; and high C-peptide levels in Susan, Candi, and Isaac.

Students could also pursue one of the cases in the videos or newspaper articles.

Most of our students found solving the cases enjoyable. They liked the real-world aspect of the cases and found that the activity allowed them to apply the information they had just learned.

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