Using a Thyroid Case Study and Error Plausibility to Introduce Basic Lab Skills

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Abstract: We describe a 3-hour session that provides students with the opportunity to review basic lab concepts and important techniques using real life scenarios. We began with two separate student-engaged discussions to remind/reinforce some basic concepts in physiology and review calculations with respect to chemical compounds. This was followed by activities designed to have the students examine and identify a wide variety of errors that can be made when taking/prescribing medication. This ultimately led to a discussion of whether a particular error can be considered "significant". In our (teaching laboratory/medical) context, the term "significant" meant an error or mistake that demands attention, as it is consequential towards the outcome. Hands-on experience of making solutions allowed the students to critically assess and attribute error prone steps to specific techniques including: calculations (including dimensional analysis), weighing out material (accuracy in weighing and the precision limits of the balance) and the role solubility can play in making homogeneous solutions. The pipetting activity gave the students hands on and scenario based opportunities to distinguish between accuracy and precision and to identify sources.

Keywords: thyroid hormone, laboratory skills, laboratory mistakes

INTRODUCTION

Not surprisingly, many studies show that students are more actively engaged in their learning when they see real life examples. Comprehension of abstract concepts is more complete when they can visualize a real life-example of an actual application or that abstraction. For example, a scenario demonstrating the chemistry of recreational drugs helped the students to appreciate the relevance of the science they are learning (Fergus et al., 2015). Popil (2011) has reviewed how real life scenarios that provide information to be analyzed and ask open-ended questions can be used to promote critical thinking.

Currently, many of the STEM enrolled college students in the United States are interested in future careers in the health field. We realized a need for real life scenarios for students in introductory classes that would reinforce abstract concepts that involved health field related issues. Adding one or two laboratory sessions could help the students learn some basic lab techniques while reinforcing the fundamental concepts. In this lab, using real life scenarios, the students learn the importance of good lab practices and the consequences that can occur from errors due to poor lab skills.

We focused on thyroid hormone and thyroid disease because over 10% of Americans are expected to have a thyroid condition in their lifetime. Thyroid hormone dosages also lend themselves well as a subject for in-class activities. Students who want to pursue a career in the health field are more likely to become engaged in the mathematical applications of unit conversions, dimensional analysis and potential source of errors than if they simply are to calculate the answer to the problem in the book. We were also inspired by the thyroid detective case developed for teaching physiology (Lellis-Santos, et al., 2011).

Parent et al., (2010) point out that "students who are asked to generate a prescribed outcome from completing a protocol generally don't care much why they are doing each step as long as they get the "right" answer." Because of this, "students begin to believe that science is about the answer and not about the process. When aiming only for the end result students are less likely to be engaged and as such miss the opportunity for understanding of both the scientific process and underlying scientific concepts" (Parent et al., 2010). Our approach and a key part of this lab was to have the students evaluate what types of errors are likely to occur and which type of errors is quite unlikely. Another significant contribution of this lab design is that some of the real life scenarios emphasize the importance of learning good lab techniques and the consequences that can occur from errors due to poor lab skills.

METHODS

We began the three-hour lab with two separate (but topic-related) student-engaged discussions to remind/reinforce some basic concepts in physiology and review calculations with respect to chemical compounds. We finished the lab period with activities designed to have the students examine and identify a wide variety of errors that can be made when taking/prescribing medication. This ultimately led to a discussion of whether a particular error can be considered "significant". It was explained to students that statistics has a particular use of the word "significant"; typically a difference is statistically significant if it could occur by chance less than 5% of the time. In our (teaching laboratory/medical) context, the term "significant" meant an error or mistake that demands attention, as it is consequential towards the outcome.

Discussion One, Part A: Understanding physiology of medication.

The students were divided into small groups and were given following two paragraphs. They were encouraged to have discussions amongst themselves concerning the medication dosage, to clarify the physiological response to, and the chemical nature of, the active ingredient in each tablet (active molecule versus chemical form present in tablet). The patient scenarios are based on the real-life experiences of the authors' relatives.

<u>Patient #1</u> needs to take thyroxine (a form of thyroid hormone) every day. Recently, she went to her Health Care Provider because she had been experiencing weight gain, irritability, memory problems, and constant feelings of cold. These are symptoms consistent with having too little thyroid hormone. The Health Care Provider looked at her recent prescriptions and noted that the patient was usually given a dose of 10 mg of thyroxine. Her most recent prescription contained 10 mg of sodium thyroxine. The Health Care Provider suspected this might be part of the problem.

<u>Patient #2</u> needs to take calcium pills in order to allow his parathyroid glands to recover. He went to his health care provider because he was experiencing muscle twitching. The Health Care Provider acknowledged it was consistent with low blood calcium levels but wanted to confirm that the patient was still taking his prescription. The patient said he had changed from the prescription Citracal to Tums. Tums is cheaper and over-the-counter. The Health Care Provider checked the labels and noted that Citracal' s label says the active ingredient, calcium, is present at 600 mg per tablet. The TUMS label says its active ingredient is calcium carbonate at 600 mg per tablet. The Health

Care Provider suspected not taking the Citracal might be the problem.

For each patient: Identify the chemical differences in the drugs prescribed and taken. Identify the chemically important molecule in each treatment and discuss how the physiology/symptoms of the patient could be related to the drug taken (or not taken).

Discussion One, Part B: Molecular basis for different concentrations of active ingredients

After the above discussion, students were presented with the three alternative analogies (below). Once the students had read the cases and the possible analogies, they were encouraged to have discussions within their groups to reach an agreement on which analogy fits each of the two patient scenarios they have discussed.

Analogy A: Thyroxine (or calcium) was like an apple, while sodium thyroxine (or calcium carbonate) was like an apple with an orange attached. In this case, there are more apples in the 1 kg of just apples than in the 1 kg of joined apple-oranges.

Analogy B: Thyroxine or calcium was like an apple while sodium thyroxine or calcium carbonate was like an apple with a leaf and stem. In this case, 1 kg of apples has almost the same number of apples whether or not the apples have a stem and a leaf.

Analogy C: Thyroxine or calcium was like an apple while sodium thyroxine or calcium carbonate was like an apple sitting in a large, dense pottery bowl. In this case, there are more apples in1 kg of apples than 1 kg of apples plus the large dense pottery bowl.

Discussion One, Part C: concentration of active ingredient in the medication.

At this point, the students understand the physiology of the drug with respect to the symptoms. They have identified the differences in the chemical composition of the medication taken versus that prescribed. They have also used the analogies to predict whether the dosage of the required active molecule was higher or lower than anticipated. Students are now told to calculate the number of moles of the ***active component*** in each of the tablets taken by patients #1 (thyroxine vs sodium thyroxine) and #2 (calcium versus calcium carbonate) and determine whether the change in the form of the medicine actually changed the dosage. Was there a 'significant' difference in the number of moles in the medication they were supposed to be taking, compared to the form they were taking?

Each student group presented their data and consensus opinion to the rest of the class. These were tallied on the chalkboard / white board / smart board and differences in data or opinions were discussed by the entire class to identify potential significant errors in the thought process, the calculation, or in understanding and applying the analogies.

Discussion Two: Drug dosages and micrograms vs. milligrams.

The next discussion was based on a scenario from a literature case (Narula, 2012). Students are presented with the following scenario:

A group of families went on a camping trip to a remote wilderness area. After 3 days of canoeing away from civilization, family A discovered that they had lost mom's thyroid medicine and in a panic, told the rest of the group. The mom needs her pills every day, and it would take 3 days to get back to their car to drive to a pharmacy and get the pills. Family B said their dog also takes thyroid pills, and they brought some extra.

Questions for student discussions in small groups:

- Can mom just take the dog pills?
- Does it make a difference whether the dog is very large (70 kg) or very small (5 kg)?

- If the human pills contain 50 ug and the dog pills 0.5 mg, is it OK if the campers just determine the number of dog pills required to be equivalent to the human pills?
- Does it matter how much mom weighs? Defend your answer.

In preparation for the first two activities, the students were provided the following scenario.

Two patients are suing their thyroid medication manufacturers concerning the strength/dosage of the thyroid pills they were taking. In response to this lawsuit, the company's scientists have test results that seem to confirm the amount of active ingredient in the pills that were prescribed. In contrast, the plaintiffs' consulting scientists have test results showing that the pills seem to be of incorrect strength. Some of the scientists, possibly on both sides, may have made mistakes along the way. As part of this lab, you [the students] will weigh some powders and pipet some solutions. This will give you practice doing these essential lab activities and as you do them, we want you to evaluate what types of mistakes are plausible and which ones are highly unlikely.

Activity One: Weights and Scales

Goals: For students to be able to identify sources of error that can occur when making solutions. Hands-on experience will allow students to critically assess and attribute error prone steps to specific techniques including: calculations (including dimensional analysis), weighing out material (accuracy in weighing and the precision limits of the balance) and the role solubility can play in making homogeneous solutions. Students can individually make a solution corresponding to each powder.

Students are told to perform the following tasks then answer/discuss these questions:

A. Make a solution by weighing out 1 gram of a white powder and bring that up to a total of 50 mL with water in the supplied conical tubes. When weighing, take care to be as accurate as possible; do not be off by more than 10% of the desired amount of the white powder. Calculate the error range first, and then weigh the sample using a balance). What is the accuracy of the 1 gram? What is the accuracy of the 50 mL?
B. While you are weighing out your powder, be aware of the potential for weighing and/or recording errors that may have been made by the scientists testing the various pills above.
C. Based on the solutions you did make, can you predict which white powders would still dissolve if a person inadvertently weighed out 10 times the intended amount?

Notes to instructors: Options for the white powders can be: artificial sweetener, baking soda, calcium chloride for canning, citric acid for canning tomatoes, white corn meal, white flour, and/or table salt.

Additional Questions for students

- One cross-examining lawyer thought a scientist could have made up ten times too much, because they misread the number, misunderstood the units or had a balance that was not accurate enough. Another thought a scientist could have misunderstood 50 mg vs. 0.50 grams.
- Which of these powders dissolved at a concentration of 1 gram per 50 mL?
- Which dissolve at a ten times more concentrated solution?
- Is it possible someone might not notice whether a powder was not completely dissolved?
- How likely do you think each of these types of mistakes would be? Does it make a difference whether the scientist was experienced or new to the lab?

Activity Two. Pipetting for accuracy and precision

Goals: Students will be able to distinguish between accuracy and precision. In addition, students should be able to identify sources of error in a method. Hands-on experience will allow students to critically assess and attribute error prone steps to specific steps.

The tasks in activity two were:

A. Determine the accuracy level of a Pipetman™, that is, if the Pipetman™ is set at 1.00 ml does it deliver 1.00 ml? It is probably best to compare the P1000 Pipetman™ with a 1 ml syringe and the P200 Pipetman™ with the plastic pipet.
B. Determine if you can pipet the same amount (within 10%) 3 times in a row, that is, how precise are you?
C. Determine whether pipet droppers are as precise as the Pipetman™.
D. Determine whether 1 ml syringes are as precise as the Pipetman™. Are the syringes accurate?
E. Imagine you have to use one P-1000 Pipetman™ or plastic syringes to pipet 1.0 ml of solution A and then 0.3 ml of solution B. Which one allows you to change volume faster? Are both within 10% accuracy?

Questions for students to consider before going on to activity three.

- Are the plastic droppers or syringes as reproducible as the expensive pipets? What is the volume of one drop? Can you deliver the same volume 3 times in a row?
- FYI, the disposable droppers or syringes cost less than just the tips for the pipets; the pipets themselves cost about $250. Do you think that it is a "good" use of taxpayer dollars (or of your tuition dollars) to have every school buy pipets? Why or why not and under what circumstances?
- Could a scientist accidently use a P20 instead of a P200? A P200 instead of a P1000? How likely is this to happen?

Activity three: Creating and evaluating the unknowns, relate to physiological response.

Goals: In addition to the chemical and physiological understanding the students gained above, we thought it was important for the students to realize that symptoms related to thyroid problems and/or thyroid medication levels can be difficult to distinguish. The symptoms could ***correlate*** with a change in medication but may not be ***caused*** by the medication. Someone with normal thyroid levels could easily gain weight, be irritable and have been having trouble remembering everyday tasks, especially if they were stressed. While rapid

Table 1: Simulated blood sample preparation

	B2 stock	purple or blue food coloring stock	How much water so the tube has 5 mL total?	What is the B2 dilution?
High purple	600 ul	600 ul		
High blue	200 ul	500 ul		
Normal purple	200 ul	600 ul		
Normal blue	100 ul	500 ul		
Low purple	50 ul	600 ul		
Low blue	25 ul	500 ul		
Your unknown				

heartbeats, nervousness, weight loss, and trouble sleeping can be signs of too high thyroid levels, the stress of college life and reliance on caffeine could also account for those symptoms.

In this section, students (in groups) created one simulated blood sample (their choice, using Table 1). Working in groups, students generated a series of controls and also assayed their unknown (made by another group). The unknown was compared to the controls to identify whether a patient had too little thyroid hormone, too much thyroid hormone or the right amount of thyroid activity.

The students also read and analyzed the data in the 2 scenarios below, being able to decipher from the data provided who had a physiological disorder caused by hormonal imbalance and who has similar symptoms that are not related to their thyroid activity.

Scenarios:
A mother brings her two teenage daughters, Abby and Beth, into the health care provider's office worried about the wellbeing of her family as a whole because both of her daughters seem to have all of the same health problems. They are both having extreme weight fluctuations and are very fatigued and can't seem to even remember what they had for breakfast that morning. Blood tests were ordered. Based on the thyroid hormone blood levels, the health care provider concluded that Abby's symptoms were due to low thyroid gland thyroid hormone production and the Beth's symptoms were caused by her acne medicine.

Eric and Frank, two 19-year-old college students go to the doctor together after discussing similar symptoms that are making it very hard to keep up with their vigorous and stressful lives. They are both in their second year of very difficult classes at college and wonder if it is all caused by stress but would like to know for sure. They are experiencing rapid heartbeat, nervousness or anxiety, trouble sleeping and hand tremors. Blood tests were run. Based on the test results, the health care provider decides that Eric's symptoms are due to an overly active thyroid gland, but otherwise a normal feedback loop. Frank's symptoms are due to taking Adderall. What would the health provider have found as values for Eric's thyroid hormone levels? What do you predict were the results for Frank's thyroid hormone levels?

For this activity, we used vitamin B2 fluorescence to mimic the fluorescence one would measure for an immunoassay for thyroid hormone. In order to hide the vitamin B2 color, the students had the choice of using either purple or blue food coloring. The tasks in activity three were:

- Students in each group made up 3 control samples using the table below, labeled H, N, and L. They also made up one additional sample, the same as one of the controls as the patient's sample and labeled it U.
- Students received a set of controls and the unknown sample from another group. They assayed the 3 control samples in parallel with the unknown using a blacklight.

Questions for students to answer:
From your samples that were assayed:

- Was your unknown high, low or normal hormone activity? How do you know?
- Which patient(s) could have provided the sample? Why?
- Was the blue or purple stock solution diluted more?
- Would it be possible for a scientist to pipet a wrong amount by misreading a row?
- Is there a better way to construct the table/instructions to reduce the chance of a mistake?

➢ Significance has different meanings in statistics and in lay use. In this lab, significant error was used in the sense that it is likely to have made such a difference in the amount of medication that a person would feel symptoms.

- For most drugs, the ability to have an effect depends upon the concentration, which is the ratio of the number of molecules (moles) to the amount of solvent.
- Units are very important. If a hamburger costs 500 would you buy it? What if it were 500 cents? The little 9 on the gas prices are mills with 1,000 mills in a dollar. The U.S. used to have tokens worth one mill (https://en.wikipedia.org/wiki/Mill_(currency)#United_States). If we still used the term mill, we might call a gas price of $2.99^9 as 2 dollars and 999 mills. If we skipped cents and converted between dollars and mills, we would probably make fewer mistakes converting milligrams to grams.

Below we have a list of likelihoods and a list of possible errors. From your experience, how would you rank the likelihood of the following errors? Might others disagree? Can you understand why there might be disagreements? What other possible mistakes did you discover were possible? What other types of theoretical mistakes the opposing lawyer might suggest did you discover that you think are almost impossible or highly unlikely?

- Almost impossible
- Highly unlikely
- Unlikely
- Possible
- Plausible
- Likely
- Very likely

- Not notice that a powder is not dissolved
- Misrecord a scale reading, such as writing down 50 mg for 0.5 g
- Misread or miswrite mg and ug or ml and ul
- For a pipet to be accurate but not precise? precise but not accurate
- Make significant errors when using a syringe instead of a Pipetman™ for delivering a set amount of volume
- Use a P1000 instead of a P200
- Use a P200 instead of a P100
- Misread the amount to be delivered on a Pipetman™
- Misjudge fluorescence amounts

Summary activity

While the students found these discussions and activities interesting and were actively engaged in all the activities, in retrospect, we feel that providing a summary of the expected learning goals or major concepts to them in advance of the exercise would probably have been helpful to their understanding of why they were doing these exercises. One has to be careful with the objectives and learning outcomes: we are not trying to dictate answers. Rather we want the students to examine their own methodology and assess the process, not just “get the right answer”.

DISCUSSION

Our laboratory training exercise focused on examining the types of errors that can occur in the lab setting. There are a number of references that suggest ways to train people to avoid pipetting errors (Epstein et al., 2003), but as far as we can determine, our exercise is the only one to consider an analysis of the types of errors that can occur in training novice students in lab techniques. Certainly, many have promoted the examination of errors as a didactic advantage. For example, textbook errors can be used as an advantage for teaching (Binder, 1984).

We have found that a combination of questions with clear right answers and questions that ask the students opinion is helpful to have in the discussions. Some students are uncomfortable and annoyed if none of the questions have a clear correct answer and other students become so focused if all the discussion questions have correct answers that they miss the big picture. In addition, most students respond very positively to being asked their opinion, but they need to learn to justify a reply with 'facts' or an understanding of process and methodology.

In this revised version, implemented with a new groups of students, the analogies in Discussion one, Part B are consistent in that, in all 3 cases, an apple is analogous to the therapeutic chemical. In the original version, each analogy (see footnote) had different objects and some students found this confusing. When students were given both sets of analogies, all students (9 out of 9) preferred the apple analogies, though they were ok with the original analogies. This discussion led a few of the students to suggest that they could come up with better analogies. This led to a great discussion and the students recognized that different students preferred different analogies. For example, one student suggested an ant as the therapeutic chemical because an ant can carry ten times its weight. Another student did not find that helpful because they didn't have a sense of things so small, and she suggested monkeys and bananas. As you might expect, an analogy generated by a student was more effective for that student than analogies by the instructor or peers and obviously constructing an analogy involves more active and critical thinking than evaluating another's analogy.

In discussion one, we have tried it both with and without supplying the molecular weights. Particularly for students with little or no background, we found it effective not to include molecular weights. They then based their answers to the sodium thyroxine and calcium carbonate cases on nebulous factors. When given the analogies, they started to make argue about which scenario matched which analogy and the discussion led them to "discover" that they need to know the weights. One that had not yet had chemistry asked, how much does calcium and carbonate weigh? This allowed others to explain the concept of moles. We have found the word moles often makes the students think about the small burrowing animals and lose track of the chemistry. In addition, 6 x 10^23 is pretty daunting to some. We prefer to introduce the idea of a set number of molecules and ask them if they understand the terms kilobyte, megabyte and gigabyte. Then we say that a mole is 600 zetamolecules and they seem to grasp that more easily than scientific notation.

Most of the students liked having real world thyroid related cases. One commented that he or she was particularly engaged, because he or she had a thyroid problem, and another said that they had a friend who had thyroid problems.

Rather than having the instructors make up the unknowns, groups made up the unknowns for peer groups. Many thought made the lab more interesting than if the instructor made up the unknowns, using words and phrases that included: "effective", "interesting", "fun", "gave more insight", and "made me feel like a mini scientist". We have found that students enjoy examining the tubes with a black light. By giving them a choice of using a purple or blue solution, some groups choose purple and see a clear yellow fluorescence and other choose blue and see a green fluorescence and allows them to think about why that occurs.

In summary, engaging the students in a health-related problem helped them appreciate the importance of chemistry and of learning proper lab techniques. In addition, most students enjoyed observing fluorescence and making up unknowns for another group. This lab activity also improved their appreciation of the types of errors that can occur in a lab and the importance of understanding basic chemical concepts and techniques in order to avoid these errors.

Footnote: The original analogies were:
Original Analogy #1: Thyroxine or calcium was like a blue LEGO brick, while sodium

thyroxine or calcium carbonate was like a blue LEGO brick with a green LEGO brick attached. In this case, 1 kg of green and blue LEGO bricks has fewer green LEGO bricks than a 1 kg of green LEGOs bricks.
Original Analogy #2: Thyroxine or calcium was like an oatmeal cookie and sodium thyroxine or calcium carbonate was like an oatmeal cookie with raisins. In this case, 1 kg of oatmeal cookies has almost the same number of oatmeal cookies whether or not the cookies have raisins.
Original Analogy #3: Thyroxine or calcium was like a robot, and that sodium thyroxine or calcium carbonate was like a robot with a very heavy backpack. In this case 1,000 kg of robots has a lot more robots than a 1,000 kg of robots wearing heavy back packs.

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