Title: The *No Quick Fix* Fix: Integrating biotechnology and hands-on lab activities into a packaged curriculum

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Abstract

This action plan proposes to add a structured biotechnology experience to a packaged problem based learning (PBL) curriculum to enhance the student's learning experience. The material is an important part of the 7th grade science curriculum adopted by Pinellas County's magnet program for gifted learners. Using the *No Quick Fix* curriculum as a foundation, several key components focusing on laboratory technique and hands on experimentation were added to increase student engagement and provide authentic learning opportunities. Material and support from the University of Florida's ICORE program provided the opportunity to expand and improve the curriculum package, and adapt it specifically to DHMS's gifted magnet program.

Background/ Rationale

No Quick Fix, published by the College of William and Mary's Center for Gifted Education, is a research based, problem based learning unit designed specifically for high ability students. The unit is structured around a ill-defined, real world problem designed to allow students to develop an understanding of the concept of systems, and to design and conduct authentic scientific experiments while learning about cell and tuberculosis biology.



The students are cast in the role of the health department investigating and attempting to control a potential outbreak of tuberculosis. The teacher's role in the

scenario is to facilitate student learner and provide pieces of the puzzle, but the unit is designed to be student directed.

In implementing the unit for the first time during the 2010-11 school year, its overall value became apparent. Some initial adjustments were made, including creating story elements that were more realistic products and presenting the unit almost entirely on a virtual platform, but I found several key elements missing from the curriculum. One of the major problems I encountered was the primary lab element published in the curriculum proved to be impractical, and the unit ended up lacking a true scientific or hands-on laboratory experience.

The ICORE institute on emerging pathogens provided me with the opportunity to address two critical concerns that I had about the No Quick Fix curriculum. First, I was able to expand my own background and understanding of key concepts in biotechnology and infectious disease. Second, it allowed me to create and implement several key components to the unit that will bring in cutting edge research and biotechnology technique. It is my hope that increasing student engagement in the scenario and providing them with a more hands-on technical experience will allow them to really exercise their critical thinking and problem solving skills.

Description of teaching unit or modules, including expected outcomes

The unit is implemented as a continuous block, and continues for about 5 weeks. I will describe the general flow of the unit, with more detailed explanations of the modules I will be adding in or enhancing as part of this action proposal.

The unit opens by briefly introducing the concept of epidemiology, and by "hiring" students as the new crisis team unit of the Eastport health department. I found that the unit is more meaningful to students that buy into the role playing element of the unit, so I have invested considerable effort in creating authentic artifacts, and building up the scenario. The materials, assignments, and research resources for the unit are almost entirely virtual, so students are able to experience the scenario in a format that more closely approximates real world problem solving than traditional text and paper assignments.

Students are immediately presented with the problem, a student tests positive for TB at the local high school. The students, as the health department, are charged with developing an action plan to contain a potential outbreak. They must decide what information they need, locate appropriate resources and research the answers, and are asked to use various reasoning models to develop their understanding of the immune system and tuberculosis. They explore tissue samples and use an online histology atlas to interpret and label what they see. At this point, students should have a basic understanding of the disease and the ways in can be transmitted.

ICORE Module 1: Interview with an Expert

A key component of the gifted frameworks in Florida is developing questioning skills in students. In this module, students have access to an expert at UF in tuberculosis and/or public health. Students will participate in a virtual conference, incorporating technology and access to authentic scientific research. The expert will offer a brief presentation or overview of TB and its impact (15 minutes), and the remaining time will be open for students to answer questions. As it may not be possible for all classes to participate in a session, the interview will be recorded. In a class session before the interview, we will discuss both questioning techniques and brainstorm the information that is needed before students develop an action plan to deal with the outbreak. Students will also prepare their questions in advance, and will be able to rank on the most relevant and important questions from all classes to present to the expert. Access to UF's experts would not have been possible without the support and connections developed through the ICORE program.

ICORE Module 2: Simulated ELISA (extended lesson plan available)

At this point in the simulation, students will have requested information about other possible TB infections, and the health department will have conducted screenings at schools and institutions that may have had contact with the initial patient. Instead of simply giving the students the test results, I wanted to have the students conduct some of the testing and analysis of the patterns of infection.

I will create a simulated ELISA test, modeled on an authentic test designed to screen patients in the field for active TB. I will incorporate Dr. Chuck Lawrence's idea using black light markers to create a simulated ELISA test. Some of the materials I need to create the simulation have been donated by ICORE, and I will purchase the remaining pieces with the classroom grant included in the project.

The ELISA will be designed to shows that some of the patients that screened positive on the Tuberculin screening do not have active TB, while a few other cases do have active TB. At this point in the simulation, students should have some understanding of the limitations of an antibody test in determining active infection, and they should have discovered the distinction between active and latent tuberculosis. However, during the initial implementation of the curriculum I found that many students still had difficulties grasping these key concepts, and those misconceptions persisted through their final evaluation. Working with the lab should help solidify their understanding of this key component of the content.

As part of the lab, I will check out UF's micro-pipetting locker. Students will be introduced to the technique of micro-pipetting, and will complete the training lab create by CPET. The use of the colored water and design cards is a fun and visual way to practice using the equipment.

I will also create patient histories so that students can attempt to trace the path of the infection, and element that I decided to add based on the success of Dr. Paul Gibb's presentation of the Rift Valley Fever training exercise. This element of epidemiology is missing from the original simulation, and this "detective story" should be a great way to engage students in a meaningful way at a point where some of their enthusiasm for the simulation was waning last year. The exercise should encourage them to form reasonable inferences based on the evidence they have, and then incorporate those elements into their action plan.

ICORE Module 3: Communications and Public Engagement

One of the most important authentic control measures necessary in any public health emergency is disseminating information to the public in a way that controls the situation and encourages the community to act in a way that helps, rather than complicates control measures. The No Quick Fix curriculum contains a component that presents letters to the editor and inflammatory statements and asks the students to respond in a reasoned way, grounded in scientific terms, that addresses the issues. I want to expand on this to include some of Dr. Gary Clark's information regarding community based interventions. I will ask students to identify one key behavior that should be changed that would help control the spread of TB, and go through the process of creating a campaign that would actually be successful in having the community adopt the new behavior.

Experts in any field tend to feel that if they could just get everyone to know what they understand, then everyone would do the right thing because it is the only thing that makes sense. If this were the case, everyone would always go for the carrot sticks instead of the french fries, as we have all been educated that the former option is healthier. Companies like McDonalds understand how to manipulate behavior, where the experts do not. Gifted students in particular have difficulty understanding other perspectives, and I think that this will be a valuable exercise in thinking outside the box to reach a desired outcome.

ICORE Module 4: Antibacterial Resistance/ Experimental Design

The original No Quick Fix curriculum includes a component where students create and execute an experimental design to determine the cleanliness of the school. Due to district regulations, and legitimate concerns about culturing unknown bacteria, I removed this element from the curriculum. The unit does add a twist to the scenario by adding the possibility that the initial patient was exposed to antibiotic resistant TB. Students must then think about the implications of resistant bacteria, and investigate measures that would be necessary to deal with MDR-TB.

I intend to bring back in the element of experimental design and charge the students with determining whether a bacterial sample is bacterial resistant or not. I will use the classroom grant to purchase a antibiotic sensitivity kit from Flynn Scientific. The kit provides the agar, petri dishes, and antibiotic disks needed to test E. Coli for antibiotic sensitivity. I will give students the materials, but will not give them lab instructions so that they have the opportunity to work out their own design.

Extension

As a follow up to the material presented in No Quick Fix, I also intend to incorporate UF equipment lockers during our unit on genetics that follows later in the year. I would like to check out the electrophoresis locker/pipetting lockers to compare an unknown DNA sample to two "suspects".

Data collection techniques and student assessments

Pretest/ Postest Fowler Science Processes Assessment (The diet cola test)
Problem Logs (student journal responses assigned throughout unit)
Lab reports

Histology Lab ELISA Lab & epidemiological analysis Antibacterial Resistance Lab

Formal action plan proposal (group)

Unit Assessment

Written: No Quick Fix Exam

Oral: Students will present recommendations to the "school board" on how to prevent a future TB outbreak.

Student feedback survey

Budget

From Flynn Scientific:					
FB1935	Antibiotic Sensitivity Testing Student Laboratory Kit	\$99.80			
LM1006	Escherichia coli. Variably motile rods found singly, paired, and in short chains. Common intestinal organism. Lactose fermenter.	\$8.75			
From Carolina Biological Supply:					
215232	Microcentrifuge Tubes Mixed colors, Pack of 1,000	\$43.95			
215575	Micro Test Tube Rack	5 @\$17.25			
From Blacklight.com:					
BLWPEN	UV Blacklight Reactive Invisible Ink Pen/Marker	3 @ \$3.99			
Code10	Code 10 Handheld Security 6 Inch Blacklight	5 @ \$7.99			
	250.75				

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TITLE: Testing for TB: Using a simulated ELISA test to determine active vs. latent TB as part of a long term simulation

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SUBJECT: Comprehensive Science/ Biology

LEVEL: Grade 7, High ability/ Gifted Students

KEY CONCEPTS ADDRESSED: Biotechnology, Laboratory Technique, Human Immune Response, Pathogens and Disease

Transmission, Immunodeficiency

OVERALL TIME ESTIMATE: 3-4 days, as part of a 6-week long unit

UNIT OVERVIEW & BACKGROUND: This lab activity is to be included in a unit called "No Quick Fix" published through the Center for Gifted Education at the College of William & Mary. The unit is a problem based unit that has the students playing the role of the health department during a TB outbreak and is designed to challenge high ability students to think critically. This lab has been developed as an extension to the original unit to introduce an additional hands-on experimental element and biotechnology technique. At this point in the simulation, students have been introduced to the initial problem, and are using problem solving models to determine a solution. The scenario begins opens when a student (Todd Smith) at Eastbridge High has been diagnosed with active TB. The health department crisis teams (the students) have been called in to determine the appropriate actions needed to prevent a widespread TB outbreak. In earlier class sessions, students have determined the need to test all individuals that may have been exposed to TB through contact with the original patient. Health department "minions" have conducted widespread TB skin testing at three local schools. Several individuals will test positive on the skin test, but may or may not have active TB.

LESSON SUMMARY: In this lab, students will take samples from these individuals and use a simulated ELISA to test for the presence of the TB antigen, indicating active tuberculosis.

NATIONAL SCIENCE EDUCATION STANDARDS ASSESSED:

- Students will be able to think and analyze data in terms of systems.
- Students will develop an understanding of organisms and environment.
- Students will develop an understanding of structure and function in living systems.
- Students will develop an understanding of personal health issues.
- Students will be able to ask questions about objects, organisms, and events in the environment.
- Students will be able to plan and conduct a simple investigation.
- Students will be able to employ simple equipment and tools to gather data and extend the senses.
- Students will be able to use data to construct a reasonable explanation.

• Students will be able to communicate investigations and explanations.

LEARNING OBJECTIVES:

- 1. Students will be introduced to micro-pipetting and controlled laboratory technique. They will focus on avoiding cross contamination and on dealing with potentially hazardous samples.
- 2. Students will be introduced to the concept behind ELISA testing, and will understand the relationship between antibodies and antigens. They will be able to describe how the two react, and why the ELISA test can determine active TB where a tuberculin antibody test cannot.
- 3. Students will clearly understand the concept of latent versus active disease, and will recognize that the presence of antibodies does not necessarily indicate an active infection.
- 4. Students will be able to explain why samples from immune-deficient individuals with a negative tuberculin test were also included in their samples to be tested.
- 5. Students will discover patterns in the data they generate and will draw inferences about how the disease may have been transmitted from the original patient. They will also be able to explain why the patients that do not have active tuberculosis were positive on the original TB antibody test.

BACKGROUND INFORMATION:

Tuberculosis:

A basic understanding of the nature, transmission and treatment of TB is required in order to facilitate the No Quick Fix unit. A great deal of information is available online. The Mayo clinic article at http://www.mayoclinic.com/health/tuberculosis/DS00372 is a good place to start, as is the Wikipedia article at http://en.wikipedia.org/wiki/Tuberculosis.

For this lesson, it is important to know that TB can only be transmitted by people with active—not latent—TB. About 90% of those infected with Mycobacterium tuberculosis have an asymptomatic, latent TB infection, with only a 10% lifetime chance that a latent infection will progress to TB disease. However, if untreated, the death rate for these active TB cases is more than 50%.

Generally, casual contact with a TB carrier does not result in infection, and infection is not transmitted from surface contact. Immuno-suppressed individuals are more likely to become infected with TB than those with a healthy immune system. Prolonged, close contact with a TB patient can result in transmission. Research also indicates that improperly disinfected swimming pool water can be a source of TB infection.

The probability of transmission from one person to another depends upon the number of infectious droplets expelled by a carrier, the effectiveness of ventilation, the duration of exposure, and the virulence of the M. tuberculosis strain. A person with active but untreated tuberculosis can infect 10–15 other people per year. When people suffering from active pulmonary TB cough, sneeze, speak, sing, or spit, they expel infectious aerosol droplets 0.5 to 5 µm in diameter.

A single sneeze can release up to 40,000 droplets. Each one of these droplets may transmit the disease, since the infectious dose of tuberculosis is very low and inhaling fewer than ten bacteria may cause an infection. However, it is people with prolonged, frequent, or intense contact that are most at risk of infection, with an estimated 22% infection rate. If someone does become infected, then it will take three to four weeks before the newly infected person can transmit the disease to others.

Outbreaks can be contained by isolating people with active disease and starting effective anti-tuberculosis therapy, which is, in most cases, long term antibiotic treatment. After two weeks of such treatment, people with non-resistant active TB generally cease to be contagious.

Active TB can be a problematic diagnosis, but administering unneeded antibiotic treatment may increase the prevalence of drug-resistant forms of the bacteria. It may take 4 to 12 weeks for blood or sputum culture to show evidence of the bacteria. A complete medical evaluation for TB must include a medical history, a physical examination, a chest X-ray, microbiological smears, and cultures. It may also include a tuberculin skin test, which yields a delayed hypersensitivity type response to an extract made from M. tuberculosis and only diagnoses latent TB infection. Those immunized for TB or with past-cleared infection will respond with delayed hypersensitivity parallel to those currently in a state of infection, so the test must be used with caution, particularly with regard to persons from countries where TB immunization is common. Tuberculin tests also produce false negatives, especially when the person has a compromised immune system due to immuno-suppresant treatments, AIDS, sarcoidosis, Hodgkin's lymphoma, or malnutrition.

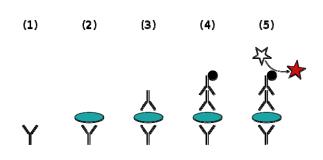
What is an ELISA test?

Enzyme-linked immunosorbent assay (ELISA), also known as an enzyme immunoassay (EIA), is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality-control check in various industries. In simple terms, in ELISA, an unknown amount of antigen is affixed to a surface, and then a specific antibody is applied over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme can convert to some detectable signal, most commonly a color change in a chemical substrate.

What is the Clearview ELISA for TB?

http://www.clearview.com/tb_elisa/technical_information.aspx

This is a real ELISA test that is currently marketed in places like Africa where TB is rampant and hard to test for because of the prevalence of immune-suppressed AIDS patients. The Clearview ELISA tests a patient's urine for the presence of the TB antigen and can determine whether a patient has active TB in the field by a technician. This is a "sandwich ELISA" that detects the presence of the antigen in the sample, rather than the antibody.



A sandwich ELISA. (1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by enzyme to detectable form. (http://en.wikipedia.org/wiki/ELISA)

Antibodies adsorbed on the ELISA plate capture the carbohydrate surface antigen found in positive test samples. The conjugated antibodies then attach to the captured antigen creating a sandwich assay. In the presence of the color developer, a color change occurs. The assay reaction is stopped using the stop solution, and the intensity of the color (optical density) is measured using a microtiter plate reader. A positive result indicates that LAM antigen of mycobacteria is present in the sample, whereas a negative result indicates that it is not present at or above the test's detection limits.

The Clearview TB ELISA does not detect latent TB infection, but is designed to detect active TB by Clearview TB ELISA specifically detecting LAM antigen derived from bacteria in the patient's blood which have been metabolized by the kidneys and passed into the urine.

ADVANCE PREPARATION:

Patient Histories:

In the ongoing No Quick Fix scenario, the health department's "minions" have tested all individuals that may have been exposed to the primary patient with active TB. The results of the school wide screening tests are given to the students on day 2, noting that 20 individuals have come back with a positive tuberculin result. Each lab team will also be asked to test an immune-suppressed individual with a negative tuberculin result that needs to be verified.

Basic patient histories for each positive test must be written that include some clues as to why some of the individuals may have a false positive and some connection to the original patient for those that do have active TB. Not all of the patients in each lab group's samples should be positive for active TB, and the students should be able to provide a possible explanation of how those individuals are connected to the original patient. These clues should be sufficiently buried in the patient history so that the connections are not immediately obvious.

Patient Histories				
False Positive (No TB)	Possible False Negative	Infected		
3 per lab group, 15 variations	One per lab group	One per lab group		

for class		
Received BCG vaccine as a child	Guidance counselor undergoing	On swim team (X6)
	chemotherapy (infected, worked	
	with Todd to fix schedule issue)	
Born in India/ South Africa	HIV-positive teacher	Todd's girlfriend
Worked as a nurse in the inner	Student taking	Todd's girlfriend's tutor
city before moving to Eastbridge	immunosuppressant drugs	
	following heart transplant	
Peace Corps volunteer in Africa	Anorexic student	
Volunteers in a nursing home	School secretary being treated	
	for breast cancer	
Had TB as a child		
Family member was exposed		
several years ago		
Homeless for a few years in the		
past		

Creating the simulated ELISA:

For each lab group, a well plate and well plate card for the ELISA test must be prepared in advance. The well plate card should be laminated and labeled clearly with a positive control, a negative control and 3 sample wells with a number that corresponds to a patient history. This card is to ensure the students fill the correct wells. To make this a guaranteed result simulation, the "enzyme" that indicates the presence of the antigen will be visible only under black light. Each well plate must be marked with black light paint added to the wells that correspond with the positive patient histories in advance. This should not be obvious to the students on inspection and, if the students are going to believe the simulation, all care must be take that they fill the correct wells of their plate and that their patient histories correspond to the plate they work with!

In addition to the pre-marked ELISA plate, each group needs 5 vials of yellow water to represent the urine samples, a green positive control, and a blue negative control.

To read the plates, a black light should be installed in black-lit box so that students can bring their filled plates and see which results glow positive.

MATERIALS NEEDED: (For 5 lab groups of 4-5 students)

ICORE equipment:

- Micro-pipetting locker, including pipettes and tips. There need to be enough tips that students can discard them during the lab, but they can be retrieved and reused if necessary.
- Hazardous waste bags will help add to the believability of the simulation.

Teacher created materials:

- Patient histories
- 5 ELISA well plates marked with black light paint
- 25 vials of yellow liquid
- 5 vials of green liquid
- 5 vials of blue liquid.
- Reserve liquids to top up vials between labs.
- Black light installed in viewer box.

Consumable materials:

- Gloves
- Paper towels
- Q-tips (for cleaning well plates)

PROCEDURES/ASSESSMENTS:

Day 1: Micro-pipetting Training

- Open with journal question/ discussion (5 min)
 - Q: How do you measure liquids? What is the smallest amount that you can measure?
 - A: Students should suggest using graduated cylinders and eyedroppers. Some may have used glass pipettes. They should recognize that it is difficult to measure less than a drop accurately.
- Direct Instruction: Teacher will demonstrate proper pipetting technique, and will explain the importance of discarding tips between different samples to avoid contamination. Also, class will discuss how and why hazardous/ biological waste needs to be discarded. (10 min)
- Student Practice: Students will work in their lab groups (5 groups of 4) to complete the micro-pipetting practice exercise. Each group will have a protocol to remind them of pipetting technique, and a lab card indicating precise amounts of colored liquid for each well. (20 min)
- Assessment: If successful, students should recognize the pattern that they were building from their lab card.
 Students will self-assess their technique and turn in a paragraph critique of the exercise. They will create a
 "reminder card" to use in the lab tomorrow to help them remember the parts of micro-pipetting technique that
 they felt most likely to forget (e.g. Set volume FIRST, Change tips between samples, Don't Pump Pipetter) (5
 min/ Homework)

Clean-up (10 min)

Materials Required:

- ICORE Micro-pipetting locker, including tips and pipettes
- Prepared pipetting protocol cards
- Multi colored water samples

Day 2: Introduction to ELISA testing

- Open with journal question/ discussion (10 min)
 - Q: What does the tuberculin test show? Does a positive tuberculin test mean that a patient is sick or contagious? Does it always work?
 - A: Tuberculin tests indicate that a person has TB antibodies in their system. It does not mean that they have an active infection, just that at some time they were exposed to the bacteria. People that had the TB vaccine will show up positive, even if they have not been exposed to the actual pathogen. Some people that have a suppressed immune system will not produce antibodies, so even with an active infection may not show up as positive.
- Whole Group Discussion (15 min)
 - As necessary, teacher will review the difference between active and latent TB, and the nature of the tuberculin antibody test with students. Students will then be informed of the results of the school based tuberculin testing and will be asked what needs to be done with these individuals. Students should determine that they need to be tested for active/infectious TB before being allowed back at school, but should understand that not all of the people with a positive tuberculin test are actually sick or infectious. We will also discuss why treating everyone with antibiotics might cause drug resistant TB, and cause a greater threat to public health. Antibiotic resistance will be further discussed in a second laboratory component later in the unit.
- Clearview ELISA briefing/ training (20 min)
 - o While in reality, these patients would probably be given a chest x-ray and a sputum test by a physician, the teacher will suggest that the health department field test the Clearview ELISA test for TB. The teacher will introduce the concept of an ELISA test, and will go over the design and procedures for the ELISA test using a PowerPoint presentation.

Materials Required:

- Scenario results from tuberculin testing/ patient histories
- Clearview ELISA materials

• Training PowerPoint describing ELISA process and lab protocol

Day 3: ELISA Simulation

- Review lab protocols (5 min)
- Students will receive their lab protocols, their micro-pipetting refresher guide, and samples and will work through the ELISA simulation with their lab group (20 min).
- Students will "read" the results and will determine which patients have active TB, and which may have had a false positive or have latent TB. (5 min)
- Assessment: The lab group will add notes to each patient history interpreting the ELISA results and explain why they make sense for each patient. (10 min)
- Clean up (10 min)

Materials Required:

- Micro-pipetting Locker
- Fresh tips
- Hazardous waste bag
- Gloves
- Patient histories
- Pre-marked ELISA well plates
- Patient "samples"

Day 4: Determining transmission patterns and updating the action plan

- As a whole group, students will process the ELISA data and will determine the most likely scenarios that explain how the original patient infected those that have now tested positive for active TB. (30 min)
- Once they have drawn some conclusions about how the TB was transmitted, they should update their action plans to reflect any new actions the health department should take based on the new information. (20 min)
- Assessment: This is a formative assessment that should show how both individual students and the class as a group have assimilated the information acquired during the investigation. The following points should come out of the class and small group discussion as they process the data and revise their action plans. Student understanding will be formally assessed at the end of the No Quick Fix unit through a formal written assessment/ essay test, and an authentic oral assessment in which each team presents a well formed plan to the "school board" advising them about measures they need to take to prevent future outbreaks of TB from occurring.
 - o In the scenario, students should surmise that the swim team members became infected because the chlorination system at the pool was malfunctioning, and they were exposed to Mycobacteria in the

- water. They should also conclude that the girlfriend got TB from prolonged contact (i.e. deep, meaningful conversations with her boyfriend) with the original patient, that she passed it to the study partner. They might also draw the conclusion that she may not be a particularly good girlfriend!
- They should be less sure about the guidance counselor, but should suggest that he was susceptible because of his weakened immune response.
- o They should recognize why the possible false negatives were included in the test samples, and should explain why it was so important that they were tested again.
- They should also recognize the TB is not that easily transmitted, as only 7 people out of the 1000 tested were infected.
- o They should recommend that the infected individuals be quarantined from school until they have taken the recommended antibiotics and have been cleared by a physician.
- They should recommend that the pool be shut down until completely sanitized and the disinfection system has been repaired.

Materials required:

- ELISA results
- Patient histories
- Online access to research article indicating that TB can be transmitted through swimming pools ((http://www.thewatertreatments.com/disinfection/disinfect-swimming-pool-water).

References

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