Research Simulation Case Study
Brain Eating Amoeba
Answer Key and Teaching Notes

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I. Introduction/Background:

The Brain-Eating Amoeba RSCS is well suited for use in AP level STEM classes, college level science orientation, introductory class or freshman biology course, as well as environmental science courses because this case was:

- Uniquely created to teach scientific literacy.
- Successfully used in a freshman introduction to Scientific Literacy class for STEM majors, a pre-freshmen bridge summer science program, an AP biology course and a STEM based high school summer program.
- Based on an actual interdisciplinary research publication (Muldrow, 1982).
- Part of the Scientific Literacy Center for Pre and Early STEM Majors.

In addition this case is designed to facilitate student learning the scientific research process across various STEM curriculum modalities.

II. Classroom Management:

Research simulation case study (RSCS) is a phrase created to describe a story with an educational message that requires students to solve research problems (Muldrow, et al, 2013). The stories told by these case studies are designed to have the student engage in the case study as a researcher. Students are told to take on the role of scientists and that they will be designing experimental protocols to solve a real world research problem. Students are then given background information (on the case) and asked to design experiments to solve the case. After the students have designed the experiments, experimental data is presented in the case study. The case study further suggests that this hypothetical data came from their protocols. The students are then asked to interpret and evaluate this data.

Since scientists often work in teams that form hypothesis and develop protocols as well as collect and interpret data, the interrupted case method simulates the way real scientific discovery works and thus is the best method to present the RSCS. Herreid (2005) contends that this approach is superior for teaching science due to the ability to mimic scientific decision making that occurs with incomplete data and that undergoes constant revision as more data is revealed. Using this RSCS, the interrupted case method gives students the opportunity to solve real research problems

while working in teams. It requires students to make hypothesis, develop protocols, and interpret data while challenging each other and seeing different teams offer alternative methods and solutions. White, et al (2009) purports that case studies enhance student engagement and cognitive development while allowing for the assessment of student understandings and learning. It has been suggested that the interrupted case studies are an improvement on this approach in that the student groups report their findings to the class at multiple points in the project which provides a mechanism to correct misconceptions and to provide support (White, et al, 2009).

This RSCS entitled Brain Eating Amoeba and its fourteen questions can be conducted in approximately four (4) hours using the interrupted case method. The answer key contains the answers to the fourteen (14) questions. The answer key also contains extra questions that the instructor may choose to ask as well as additional sub-case studies. The sub-case studies are interactive computer modules or case studies that teach the mathematics and/or content underpinning the questions asked in the Brain Eating Amoeba case. The Brain Eating Amoeba RSCS is designed so that upper level high school students and freshmen college students can complete it without the use of the sub-case studies; however, the sub-case studies add breath to this exercise and will help insure that each student has mastered relevant skills. Each of these sub-case studies range in length from a few minutes to several hours. Conducting the RSCS Brain Eating Amoeba along with all of the sub-case studies and extra questions can take up to 20 hours to complete. This can effectively be accomplished in a summer program. If sub-case studies are not used completion time significantly decreases. An instructor may choose which if any sub-case studies are used.

The Brain Eating Amoeba RSCS has also been designed such that it can be taught in modules. For example this case study may be used in a high school physics class in which page one (sections A and B), page 5 (sections F and G) and page 6 are given to students. These sections effectively deal with fluorescence and optics. In this case the instructor would give some background information to place pages 5 and 6 in context. Numerous other modules can be extracted from this RSCS based on the instructors course objectives.

To present this case using the interrupted case method (Herreid, 2007) students are ideally placed in small groups which work together. The teams are given a specified time to finish a question or group of related questions. After teams work on the problem, the instructor asks one or more teams to report their thoughts. If the instructor has access to clickers or a class room response system, then the instructor can ask the entire class if they agree to the reported thoughts. After showing the results to the entire class, varying opinions can be solicited and additional information provided as appropriate. The students then return to their small groups to work on the next question or group of related questions. This cycle of group work, group reporting, class validation and instructor discussion is repeated until the case study is finished.

IV. References:

1. BD Biosciences (2000) Introduction to Flow Cytometry a Learning Guide http://www.stemcell.umn.edu/prod/groups/med/@pub/@med/documents/asset/med_80691.pdf
2. CDC Print and Go Fact Sheet (2012) Facts about *Naegleria fowleri* and Primary Amebic Meningoencephalitis http://www.cdc.gov/parasites/naegleria/Naegleria_factsheet508c.pdf
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5. Invitrogen Flow Cytometry Tutorial http://probes.invitrogen.com/resources/education/tutorials/4Intro_Flow/player.html
6. Martinez, A.J. (1996) Chapter 81 Free-Living Amebas: *Naegleria, Acanthamoeba* and *Balamuthia* in Medical Microbiology 4th Edition (S. Baron editor), Galveston TX PMID: 21413280 http://www.ncbi.nlm.nih.gov/books/NBK7960/#_ncbi_dlg_citbx_NBK7960
7. Muldrow, L.L., Tyndall, R.L. and Fliermans, C.B. (1982) Application of flow cytometry to studies of pathogenic free-living amoebae. Applied Environmental Microbiology 44(6), 1258-1269.
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9. Robinson, J.P., Sturgis, J., and Kumar, G.L. (2000) Chapter 10: Immunofluorescence in Education Guide: Immunohistochemical (IHC) Staining Methods 5th Ed (George L. Kumar and Lars Rudbeck editors) pps 61-65. http://www.dako.com/08002_03aug09_ihc_guidebook_5th_edition_chapter_10.pdf
10. White, T.K., Whitaker, P., Gonya, T., Hein, R., Kroening, D., Lee, K., Lee, L., Lukowiak, A., and Hayes, E. (2009) The use of interrupted case studies to enhance critical thinking skills in biology. Journal of Microbiology and Biology Education. 10, 25-3.

III. Answer Key:

Answers are given below to each of the 15 questions in Brain Eating Amoeba RSCS. For select questions "Supplemental Material and/or Sub-Case Studies" online are suggested to assist students in answering the question. Student participation in the Supplemental Material and Sub-Case Studies is not required and is up to the discretion of the teacher.

Question #1 - Research Activities:

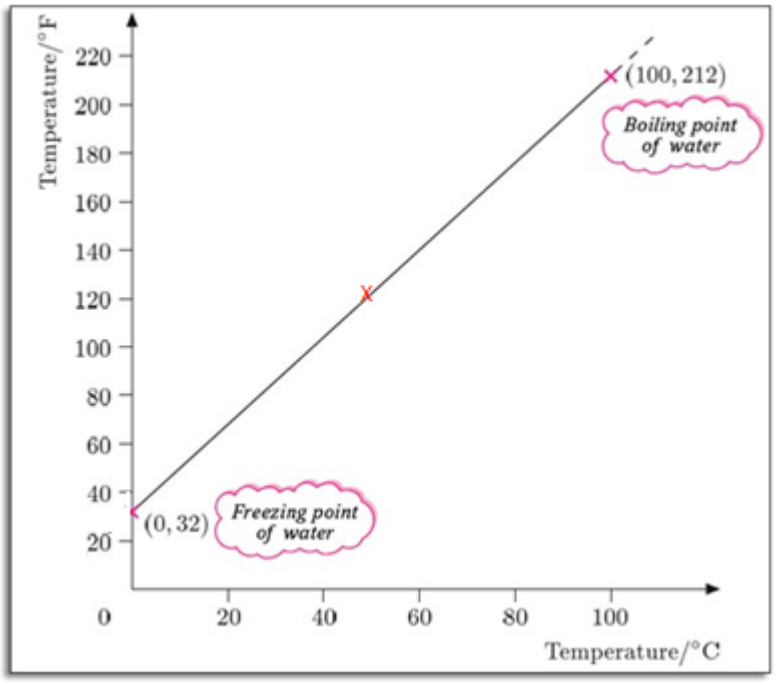
Draw a simple X-Y graph plotting degrees Fahrenheit versus degrees Celsius. Using the formulas below, convert the following temperatures from degrees ^{o}F to ^{o}C: 32^{0}F, 120^{0}F, and 212^{0}F. Draw a line through the points on the graph.

$$T_c = \frac{5}{9} \times (T_f - 32) \qquad T_f = (\frac{9}{5} \times T_c) + 32$$

T_c = temperature in Celsius T_f = temperature in Fahrenheit

Answer #1:

The answers are 32^{0}F =0 ^{o}C, 120^{0}F = 48.9 ^{o}C, and 212^{0}F =100 ^{o}C. Students should be given graph paper to graph and draw the line.



a. Supplemental Material
The following two links can assist students in learning how to solve algebra equations.
www.ixl.com/math/algebra-1/solve-two-step-linear-equations
www.ixl.com/math/algebra-1/solve-advanced-linear-equations

Question #2 - Research Activities:
Using the graph you created, extrapolate the lowest and highest temperature from each site in Table 1 on your graph. Now that you have determined the temperature ranges of your sites in ^{o}C, which of these sites would you test, or not test, for the presence of *N. fowleri* and why (be specific for each site)?

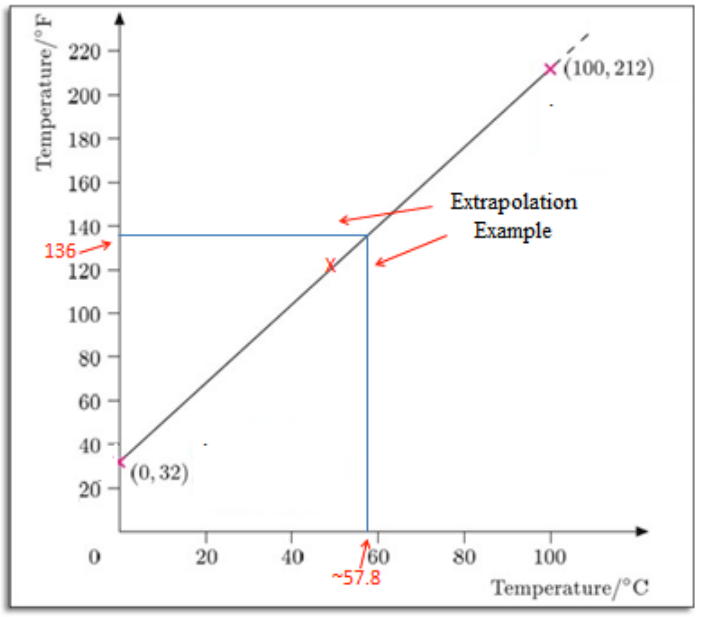
Table 1

Environmental Sites	^{o}F Range
Site 1	61^{o}-76^{o}
Site 2	92^{o}-113^{o}
Site 3	124^{o}-136^{o}
Site 4	35^{o}-68^{o}
Site 5	75^{o}-107^{o}

Answer #2:
The ^{o}C temperatures in the answer key table below have been given to the first decimal place. Students will extrapolate temperatures from their graph and their answers will not

be this precise; however, they should extrapolate temperatures within (plus or minus) a couple of degrees of the answers given below.

Site	°F Range	°C Range
Site 1	61°-76°	$16.1^0 - 24.4^0$
Site 2	92°-113°	$33.3^0 - 45.0^0$
Site 3	124°-136°	$51.1^0 - 57.8^0$
Site 4	35°-68°	$1.7^0 - 20.0^0$
Site 5	75°-107°	$23.9^0 - 41.7^0$



Site 1: You would not test Site 1 because when water temperatures are below 26.7 °C (which converts to 80°F) the amoeba, if present, would exist in the cyst form which is not pathogenic.

Site 2: You would test Site 2 because these waters are the perfect temperature for the pathogenic *N. fowleri* trophozoite.

Site 3: You would not test Site 3, the water is warmer than 50^0C and would kill the amoeba.

Site 4: You would not test Site 4 because when water temperatures are below 10°C and *N. fowleri* is destroyed. Also the temperature never rises above 26.7°C (see explanation for Site 1 above).

Site 5: You would test Site 5 because the trophozoite will grow when the waters are warmer. A side note for discussion is even though the cyst will form when the waters are cooler than 26.7 °C, the pathogen will emerge when the water is warmer.

Question #3 - Research Activities:

You are also interested in identifying at least three more potential environmental sites from a different geographic area that might contain the trophozoite form of *N. fowleri*. Describe three different natural geographic locations or thermally polluted sites you would sample. An example of a description that you might make is "a shallow river in southern Florida."

Answer #3:

Students should select sources which are warm freshwater not exposed to chlorine or salt, for example:

a. Thermally polluted water such as nuclear reactor run-off
b. Industrial run-off where the company is placing hot water in a stream, river or lake
c. Naturally occurring hot springs
d. Shallow regions of lakes in tropical or subtropical climates
e. Hot sewage treatment water before the water is treated
f. Shallow man-made reservoirs in tropical or subtropical climates
g. Any other creative sites that are warm fresh water not exposed to chlorine or other disinfectants

Students should not select sites such as the following

a. Lakes, rivers, streams or man-made reservoirs in climates that are not extremely hot i.e. temperate climates
b. Deep lakes or bodies of water that are in tropical or subtropical climates because the water temperature deep in the ground is always cool (as low as 56^{o}F) which in general cools the lake
c. Water sources that may have chlorine or other disinfectants
d. Salt or brackish waters

Question #4 - Research Activities:

Describe the method, or how you would collect samples from your environmental sites. Include in your answer how many samples you would take from each site and why.

Answer #4:

There are two different answers to this question based on whether or not the supplemental material is used. If the supplemental material in the sub-case study (see below) is not used, the students' answer to this section will vary. Any procedural answer that makes since is acceptable, such as, we will take water samples. If the instructor choses to engage students in the sub-case study the students should state that they will use the presence/absence method. In presence/absence sampling many samples from their site will be taken to determine if the *N. fowleri* is present or absent.

As it relates to how many samples they will take from each site and why, the students' answer should be based on the statement "*N. fowleri* is always found in low numbers in the environment" which appears in section "B. Life-Cycle *of N. fowleri.*" The students should state that they will take a large number of samples because the amoebas are found in low numbers. A reasonable range of samples to collect from each site is 25 to 250; however, this number is only a guide for class room discussion. If the student gives the correct reason, any reasonable number should be accepted as correct; unless the students chose an outlandish number of samples. For example stating they would collect tens of thousands of samples may not be possible because of resource practicality, i.e. money and time involved.

a. Supplemental Material: Sub-Case Study

Go to www.mathbench.umd.edu then click "Environmental Science," then click "Sampling," then complete section 6: Bare bones sampling:P/A and section 7 Collecting P/A data. You may also go to the site directly http://mathbench.umd.edu/modules/env-science_sampling/page01.htm This sub-case study will give students background information on how to collect environmental samples which includes sampling for abundance and presence/absence sampling.

Question #5 - Research Activities:

The white line in the fluorescent microscope picture (Figure 5) of *N. fowleri* is 20 micrometers (μm) in length. What is the approximate range of sizes of the fluorescing *N. fowleri* cells in μm? What is the approximate range of sizes of these cells in mm and nm?

Note that: 1 millimeter (mm) = 10^{-3} meters 1 micrometer (μm) = 10^{-6} meters

1 nanometer (nm) = 10^{-9} meters

Answer #5:

The amoeba size range is approximately 7μm to 20μm. It is approximately 0.007mm to 0.02 mm or 7000nm to 20,000nm. Note, the size range and average size are not exact; a plus or minus 5μm is acceptable. It would be useful to provide students rulers. A point of interest that is not part of this question/answer that some inquisitive students might ask is why are the trophozoites spherical? The fluorescent staining process kills the cells and they assume the most stable configuration. The following supplemental information will assist students in learning the metric system, the relative size of things and logs.

a. Sub-Case Study

Go to www.mathbench.umd.edu then click "Measurements," then click "The Size of Things," then complete this entire section from 1 through 7. You may also go to the site directly http://mathbench.umd.edu/modules/measurement_sizes/page02.htm This sub-case study will explain metric conversions.

b. Sub-Case Study

Go to www.mathbench.umd.edu then click "Measurements," then click "Basic Lab Techniques," then complete the subsection "Metric Review." You may also click on "Metric Review" after going to the site directly at http://mathbench.umd.edu/modules/measurement_pipet/page01.htm This sub-case study will give students a review of the metric system.

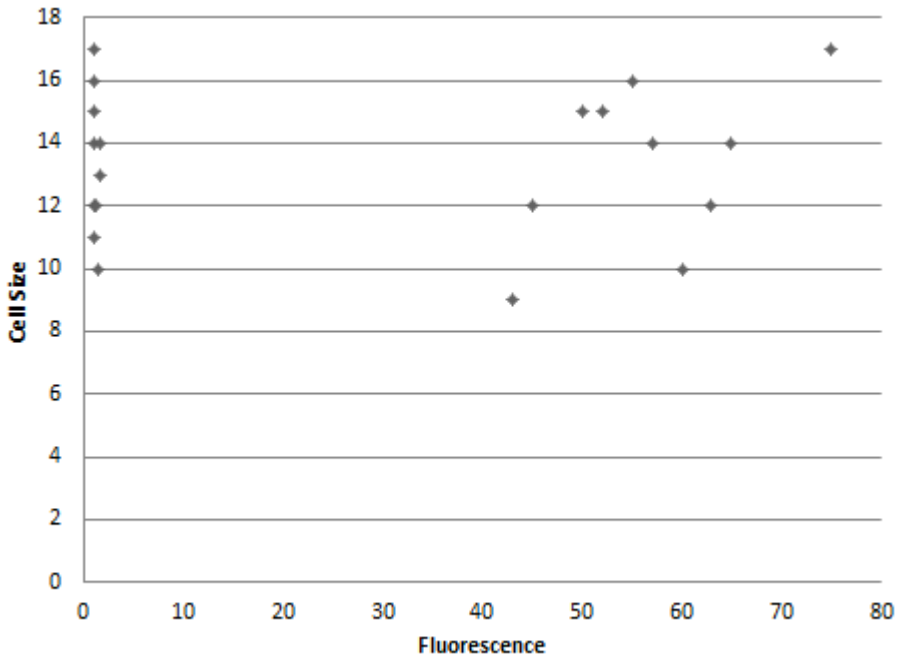
c. Sub-Case Study

Go to www.mathbench.umd.edu then click "Measurements," then click "Logs and ph," and complete numbers 1-4 in the section titled "Everything you always wanted to know about logs (but were too bored to ask)," and complete the number 12 in the "Review." You may also go to the site directly and complete numbers 1-4 and 12, the direct link is http://mathbench.umd.edu/modules/measurement_logs/page01.htm This sub-case study will give students an understanding logarithms.

Question #6 - Research Activities:

There are ten fluorescing cells and ten non-fluorescing cells in Figure 7. The cell size and degree

of



fluorescence for each of these cells is given in Table 2 below. Create a cytogram from the cells in Figure 7 by drawing an X-Y graph, with fluorescence as the X axis, and size the Y axis. Then plot a dot on this graph for each of these twenty cells.

Table 2.

Flu. Cells	1	2	3	4	5	6	7	8	9	10
Cell Size µm	17	9	15	12	10	14	16	12	14	15
Level of Flu.	75	43	50	45	60	65	55	63	57	52
Non Flu. Cells	1	2	3	4	5	6	7	8	9	10
Cell Size µm	16	14	10	13	12	14	17	11	12	15
Level of Flu.	1.1	1.0	1.4	1.6	1.0	1.6	1.0	1.1	1.3	1.0

*Flu. in Table 2 represents "level of fluorescence."

Answer #6:

Students should be given graph paper, and generate the following figure.

This cytogram depicts how data is generated from an older flow cytometer (Muldrow, 1982). Modern day flow cytometers are much more sophisticated and generate more complicated cytograms. This simplistic cytogram (size vs. fluorescence) is used because it provides graphical data within the comprehension level of freshmen and high school students.

Question #7 - Research Activities:

Which laser would you choose for your experiment? Be sure to choose a laser/light with a source emission wavelength that would generate the best excitation of the marker you are using in your experiment with anti-*N fowleri* rabbit primary and FITC-secondary antibodies. Explain in detail why you made this choice.

Answer #7:

The argon ion laser should be chosen. The FITC marker that is being used in your experiments is excited in a range of wavelengths from around 400nm to just over 525nm. The argon ion laser emits focused light in this same wavelength range as indicated by the six bars between 425nm and 500nm. More than any other laser the argon ion laser emits the correct wavelength of light to excite the FITC electron (400nm to525nm) to a higher energy level.

Question #8 - Research Activities:

Which detector would you select for your experiment and why? What wavelengths does this detector read?

Answer #8:

The FL1 detector should be selected. FL1 should be used because FITC will fluoresce in the wavelengths of around 475nm to 550nm. According to this figure the FL1 detector reads wavelengths of energy from approximately 475nm to 525nm. (Note the answer that might be given is that FL1 should be selected because it detects green light and FITC fluoresces green. This statement is correct, and students should recognize this fact; however, this answer does not correctly respond to the question "what wavelengths does this detector read?")

Question #9 - Research Activities:

Given the information in this case study, outline the simplest protocol that can quickly and easily survey for and identify *N. fowleri* in the environmental samples you have collected in the first part of this case study. Explain why you are doing each step in your protocol. Remember to add a control or two to your protocol. A control for this experiment refers to testing a control population of cells that you know are *N. fowleri.* This would be a positive control. A negative control would be a population of cells that you know does NOT contain *N. fowleri*. Using these two controls, you can compare your environmental samples to determine if *N. fowleri* is present or absent. This is done in order to make sure that your experimental protocol to identify *N. fowleri* does not mistakenly identify an entirely different amoeba or a closely related species of *N. fowleri*. *N. fowleri* is one of 45 identified species of *Naegleri*, such as *N. lovaniensis* and *N. guberi*, which are non-pathogenic.

Answer #9:

Experimental Protocol:

a. Place each environmental sample on a separate petri dish with bacteria, such as *E. coli*. (This will increase the number of *N. fowleri* cells. The amoebae will eat the bacteria, grow and multiply into a very dense population of cells, as explained in section "D. Indirect Immunofluorescence.")
b. Incubate the petri dishes at 42^{o}C. (*N. fowleri* grows fastest at 42^{0}C, and most other amoeboid species do not survive at temperatures at or above 42^{0}C, as explained in section "C. Warm Waters"). This temperature selection process will ultimately make it easier to detect *N. fowleri*.)
c. Collect the amoebae that grew on the petri dishes and treat these cells with *N. fowleri* rabbit antibodies, and FITC conjugated goat anti-rabbit antibody. (N. fowleri cells should bind to the fluorescent marker; thus, allowing for detection.)
d. Run these samples in the fluorescence-based flow cytometer. (Looking at the cells under a microscope is not the appropriate choice because the question asks

for a protocol that can quickly survey for *N. fowleri*. Flow cytometry is faster than microscopy in that it can read thousands of cells in a minute, as detailed in

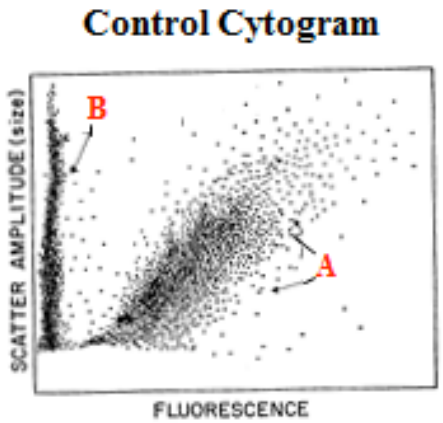


Figure 10

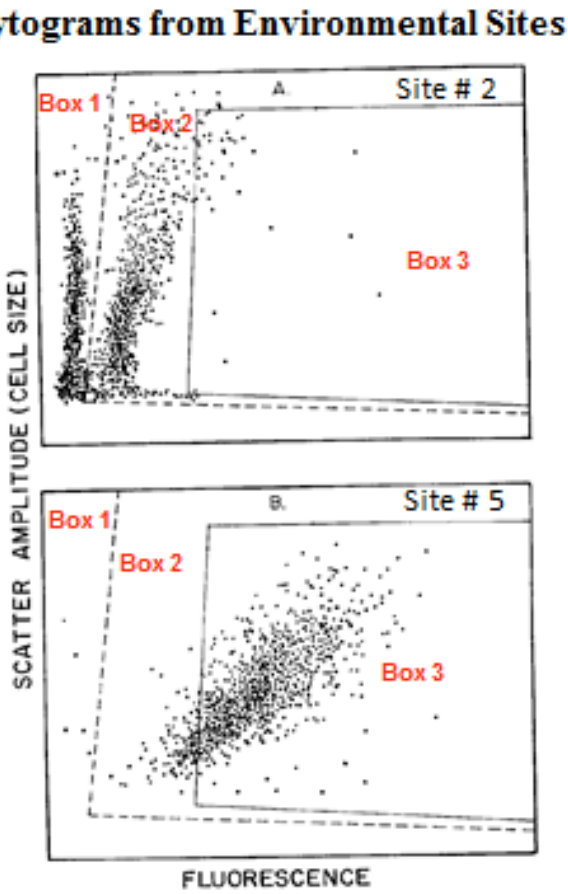


Figure 11

section "E. Flow Cytometry.")

Controls:

Students should come up with controls that only contain *N. fowleri* and a separate control that contains another species of amoeba such as *N. lovaniensis* and/or *N. guberi* as is already stated in the research activity. Students may also describe controls that contain a mix of *N. fowleri* and another species of amoeba. Then treat these controls with anti-*N. fowleri* rabbit antibodies, a FITC conjugated secondary anti-rabbit antibody, and then use the flow cytometer to analyze the cells. This will generate a graph that will allow you to see the difference between the fluorescing *N. fowleri* and another non-fluorescing species of amoeba. Additional controls in a research lab would include samples that do not have the primary antibody, or secondary antibody, etc, however, this is not important for this case study.

Question #10 - Research Activities:

In this control experiment represented by Figure 10, thousands of *N. fowleri* and *N. lovaniensis* cell were mixed. Which cluster of cells (A or B) represents *N. fowleri,* and which cluster

represents *N. lovaniensis?* Explain your answer.

Answer #10:
The cluster of cells labeled A represe*nts N. fowleri*, because these cells are fluorescing. The cluster labeled B is *N. lovaniensis,* because these cells are not fluorescing.

Question #11 - Research Activities:
In the cytogram B labeled Site #5 of Figure 11, what is your interpretation of the cluster of cells found primarily in Box 3? Do you think you have identified *N. fowleri* and why?

Answer #11:
Evidence suggests that *N. fowleri* has been found because the fluorescing cluster of cells in Site #5 appears identical to the cluster of fluorescing *N. fowleri* seen in the control.

Question #12 - Research Activities:
In the cytogram A labeled Site #2 of Figure 11, what is your interpretation of the cluster of cells in Box 1?

Answer #12:
Box 1 of cytogram A Site #2 would indicate that *N. fowleri* has not been found because the cells are not fluorescing and generating a cluster as seen in the control. (It could be proposed that another unknown species of cells that can grow at 42 ^{o}C has been isolated.)

Question #13 - Research Activities:
In the cytogram A labeled Site #2 of Figure 11, what is your interpretation of the cluster of cells in Box 2?

Answer #13:
The answer is that it cannot be determined with absolute certainty what has been found. The cluster of cells in Box 2 of cytogram A Site #2 are clearly fluorescing; however, these cells are fluorescing to a lesser degree than *N. fowleri* control cells (Figure 10), and do not display the same pattern of the fluorescing cluster of *N. fowleri* in the control.

Question #14 - Research Activities:
What could you do with the cells that generated the cluster in cytogram site #2, Box 2 to collect further data to determine if you isolated a pathogenic amoeba or not. Also, what could you do to determine, definitively, if you have isolated *N. fowleri* from the sample that gave the pattern in cytogram A Site #5 of Figure 11?

Answer #14:
Amoeba introduced into the nostrils of mice kill the mice within several days as indicated in section "A Brain Eating Amoeba." To collect further data to determine if the cells that generated the pattern in cytogram A - Box 2 may be pathogenic *N. fowleri,* you can introduce this sample into the nostrils of laboratory mice. If the mice die, then you may have *N. fowleri* or another related species of pathogenic amoeba that grows in similar warm freshwaters. To test your hypothesis that the sample that generated the cluster in cytogram Site #5 is *N. fowleri*, simple introduce this sample into the nostrils of mice. If the sample is *N. floweri* the mice will die within several days confirming your hypothesis.

Question #15 - Research Activities:
For you to be an effective scientist and solve the research problem in this case study, you had to conduct background studies in specific disciplines. Each discipline in this case study was labeled by an alphabet (i.e. A through G). Your next assignment is to identify the disciplines you studied by placing the letter of that section (A through G) in the Table 3 next to the biology, chemistry and physics disciplines. For the discipline of mathematics place a number (1-14) for the "Research Activities" that required Mathematics. Place only one alphabet or number per box.

Answer #15:

Disciplines & Descriptions	Sections A-G
Biology - Study of life sciences	
• Pathology – study and diagnosis of disease	A
• Protozoology – study of single cell organisms	B
• Environmental Science – relationships among organisms & environment	C
• Immunology – study of the immune system	D
• Cell Biology – study of cells (note "cyto-" is a prefix meaning cell)	E
Chemistry – focuses on composition and properties of matter	
• Photochemistry – chemical reactions that emit or absorb light	F
Physics – science of matter, energy, force and motion	
• Optics – science of light	G
Mathematics – focuses on relationships among numbers	
• Algebra – uses symbols like letters to represent unknown numbers	1
• Metric System – international system of units (not a discipline)	5

Table 3