

Dead or Alive -- TA Notes

WEEK 1 - Overview:

This lab is designed to give you some experience with the overall research process. Research costs time and money. Often preliminary data must be collected to establish trends and define the experimental system. In this particular lab, you are asked to determine which samples collected from various areas in a deep oceanic trench, are most likely to contain evidence of life forms. You are not asked to definitively identify each sample. The exercise involves developing operational definitions of alive, dead, organic and inorganic to use in developing your protocol, writing a grant proposal and develop a budget for the proposed research.

In the scenario presented, each lab is “competing” for the government contract to analyze the thousands of grab samples that were taken from the different areas of the deep trench. To determine which lab will receive the contract (and the funding) the government has provided each of the labs which have applied with a small subset of the samples. The contract will go to the lab which develops the most cost effective and accurate methodology for determining which of the samples contain evidence of life.

At the Beginning of Lab – Week 1:

Begin the lab with a discussion (by the students) of the following questions. A copy of the questions will be available on each student lab bench and in the lab notebook at each bench.

Allow the groups about 15 min to discuss these questions among themselves. Then bring the whole group back together and ask each group one or more of the following questions (15 to 30 min more).

- 1. What are the goals of this lab module?** Define the purpose(s) of this lab investigation.
 - as a student in Bio 151.
 - as a researcher in the scenario presented.

How real is the scenario presented? If students feel this is not real, have them explain what it is they feel is not real and why they feel this way.

Hand out samples of vials containing the types of specimens they will be asked to test. Note that the samples they are looking at this week are not necessarily those that they will analyze the following week. However, it will give them an idea of the amount of each sample available to them. They will have to consider this in setting up their proposals. That is they can't go back to the deep trench and get more sample, this is all they will have.

- 2. How is a definitive study different from a preliminary study?** What type of study is required in this case?

Here the idea is to get students to understand why they are not proposing to definitively identify each of the thousands of samples at this stage of the research.

3. What factors will you need to consider in working out your grant proposals?

Many of these are already listed as prelab questions in the lab manual. This type of information should include the following.

a) What background information do you have already?

- What exactly do you know about the sample collected? How many are there? Over what area were they collected? What do you know about the site?
- If you are awarded the grant to process all of the samples, what will your results be used for? At what stage is the research in this field?

b) What operational definitions will you use for alive, dead, organic and inorganic and any other relevant categories for your experiments? Why do you need to use operational definitions?

In this part of the discussion, have students explain how working definitions differ from complete definitions and why they would need to use working definitions in their study. The major point here is NOT that it is hard to define life, etc. Rather, what is important is development of the definitions, processes, methodologies that would provide them with the best evidence for life, etc.

c) What types of “controls” will you need in your experiments and will you need to run more than one sample of each unknown? What is a positive control? What is a negative control? Why consider replicates? Of what use are they?

One of the questions on the first pages of the lab itself asks how many tests are needed in each case. Here by number of tests we mean both number of replicates and number of different types of tests that would need to be run to determine if something is organic, alive, etc. based on the operational definitions.

d) Who will read the proposal and what are they looking for? Hint – If they already knew exactly what they wanted would they be requesting proposals?

e) What form does your proposal need to take? What belongs in the Introduction? What belongs in the Materials and Methods section? What will you do with the results after you collect them?

f) What costs must be considered in developing the proposed budget for the research and how these will affect the continued operation of your lab?

What happens if the budget you propose is too high? Too low?

Note: You do not need to include a budget in your first preliminary proposal. However, be sure to include one in your final proposal to NSF.

Question you can use to help students while they work in small and large groups on the questions above.

- 1) What is the easiest way to determine whether your sample is all of one substance or a mixture of several substances?
- 2) In determining whether something is dead and organic versus inorganic. What clues would you look for to determine which is the case? For example, do any living organisms produce inorganic compounds as part of their life cycle? (e.g. CaCO_3 shells of molluscs)
- 3) Of all the tests noted in the lab manual, one is perhaps the best for determining whether something is alive. What is it? (Hint: This tests for the presence of redox reactions. – the Tetrazolium test)
- 4) If something tests positive for alive, what methods could you use to induce greater activity or growth of the organism? Would you need to do this for this proposal?
- 5) If something tests negative for organic compounds, can you automatically assume it is inorganic. What further tests might you do to determine if any part of it is organic? (For example if you run the starch test, Benedict's test and Biuret's test on wood they will all come up negative.)
- 6) Since burning can take more than 1 hour per sample, so we need to burn every sample? What information does burning give us that other tests do not? How could we decide, relatively quickly, which samples might need burning?
- 7) If something burns is it automatically organic? (E.g. sulfur and magnesium burn)

Things to watch out for!!!

- 1) Not everything that burns contains C (light a match or two – sulfur and phosphorus)
- 2) Not everything that contains carbon is organic (e.g. pure diamond, CaCO_3 , etc).
- 3) The purpose of this study is NOT to definitively identify the exact composition of the samples, but to quickly, accurately (and at least cost) determine which samples contain evidence for life. (Note the collection site for each sample was recorded. Given this the next mission can focus on the collection sites that show evidence of life.)
- 4) In past years, we haven't seen much touching, smelling or observing of samples under the microscope. Ask questions which get students to consider how much information they can get from this. For example, alive and alive but now dead organisms should have orderly structure and evidence of cellular organization. These are not necessarily found in either free organic compounds or inorganic compounds. While a few organic compounds can crystallize in aqueous

solution (e.g. urea and sugar) most don't. As a result, crystalline structure is often used as an indicator of an inorganic compound (e.g. salt, silica).

5) Just like in nature, some of the samples may be composed of one type of stuff or more than one type (a number of the samples are mixtures).

6) Emphasize that you don't know any more about the samples than the students do.

After the large class discussion:

Small groups will reform to begin developing their preliminary proposals. These should include:

- a) A brief introduction that indicates the purpose and rationale for the project.
- b) A preliminary experimental design including a flow diagram of the procedures to be used.
- b) A plan for how the procedures will be subdivided among group members
- c) A list of supplies and equipment that will be needed to conduct the preliminary tests during week 2 of lab.

Approx. time = 45 to 60 min.

Students should peer review each others' proposals or a "sample proposal"

It's a good idea to have the students peer review a sample proposal before completing their own preliminary proposal. A sample proposal is included in this TA resource file.

As students review the sample proposal have them keep the following in mind: You are a reviewer and are responsible for allocating the money available to support this research. Good decisions advance the research. Poor decisions will bring it to a halt. What would you look for in a proposal?

Remind them also that the purpose of doing this type of peer review is to make all proposals as good as possible. For example, assume you are all members of the same research lab. You sink or swim together. You review each other's proposals to help the lab as a whole do better and hopefully to earn the million dollar contract.

Review the proposal individually (**10 min**).

Come together in small/large group to provide comments on how the proposal could be improved. What's missing that should be there, etc.? (**10 to 15 min**)

Following the peer review students will revise their own preliminary proposals and hand them in at the end of lab.

What to look out for!

Most problems noted with this lab had to do with “preknowledge” of what a specimen actually is. Providing powdered or crushed specimens is meant to overcome this problem.

The lab manual asks the students to assume that these samples are from an oceanographic expedition to the deep trench. Their tests are to be devised to determine if these specimens would be considered alive/dead, organic/inorganic based on definitions associated with life as we know it on the surface of the earth. (Ask them to consider, however, what might be wrong with this assumption.)

In large group discussion, we will probably have to remind them about dry vs wet weight and why a dry organism is usually a dead one. Use questions (as you go around the room in small and large group discussion) to clue them into the need to add water to activate potentially live but dormant specimens.

Point students back to labs 1 and 2 for methodologies generally available in this lab. Indicate that they probably won't need to use all of the, but they can. In general however, it is best to select the smallest subset of tests which taken together give the best evidence relative to the question of alive/dead etc.

Week 2 -- Students conduct their preliminary tests on samples.

Before Lab:

1) “REVIEW” THE PRELIMINARY PROPOSALS DEVELOPED WEEK 1

Write one general overall comment sheet that covers what is good and what needs to be improved for groups as a whole. (A sample review sheet is included in this TA resource file.)

Xerox the general review comment sheet and give a copy to each student in the lab.

Comments on individual proposals should be in the form of questions. For example:

- Will you need to include controls for your sampling methods? If yes, what would these be? If not, why not?
- Keep in mind the reviewers may not be entirely familiar with the study. Given this, have you provided enough information for them to understand what you plan to do and why you plan to do it?
- Under no circumstances should you attempt to outline an “appropriate procedure/flow diagram” for the students. Let them learn by trial and error.

2) PREPARE THE FOLLOWING

a) Get ice from loading dock area of Zoology Research.

(Note the back door is locked at 5 pm so do this before then.)

b) Turn on water baths – two at 37 C and two at 60C

c) If yours is the first lab of the day:

1) Mix up 500 ml of Tetrazolium solution and put in repipet jar on ice.

500 ml should last through 3 labs.

(The solution is 5 g of Tetrazolium red powder plus 500 ml dH₂O. Preweighed powder should be in the walk in cold room across from Rm 267 Noland.)

Prepare a demo of boiled vs nonboiled beans to show the tetrazolium reaction.

2) Check the fluid levels in the other repipets. Extra Biuret's and Benedicts' solutions, 1 M HC l etc should also be in the walk in cold room across from Rm 267 Noland.

3) Check the Feulgen's reagent (Schiff's reagent). It should be clear. If it has turned pink at all it will not work.

Note you can apply some of the Feulgen's to a piece of chalk (no DNA) to demo the fact that it will oxidize rapidly on such surfaces and turn purple. However, the color is not localized. Students need to look for localized color, e.g. in nuclei, to demonstrate DNA is present. To do this they will need a microscope.

4) Nutrient and nonnutrient agar plates.

- Use the plates in the walk in cold room. If your lab uses a significant number of the plates, your students will need to make more for subsequent labs. Instructions for preparing new agar will be in the lab.
- Each plate takes about 30 to 35 ml of agar solution and takes about 2 to 3 hours to set.
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d) If yours is the last lab of the day –

- Dump out tetrazolium at end of lab
- Put Feulgen's in cold room (other reagents can stay at room temp)
- Turn off water baths.
- Dump ice
- Turn off air cleaners if used.

e) All labs – At the end of lab:

- Make sure students wipe off bench tops (including around sink area) before they leave. All glassware should be clean and returned to appropriate locations. Same for solutions, etc.

Week 3 – Discussion of preliminary results and preparation of final proposals

During Week 3 of the lab, students will begin by reporting the results of their preliminary experiments. Have them put all results on a summary overhead at the front of lab, being sure to indicate group number/names as well as results.

Within each lab there should be two of each sample type distributed among the groups. During this discussion groups should compare both their results and their methods. Students will need to include all groups' results in their final proposals.

1) Have all groups list their results on the overhead (see form below).

2) Next, go through each sample like the following:

Group 4, you had sample 1 - What did you find and what did you decide as a result of your findings?

Group 5, you also had sample 1 -- - What did you find and what did you decide as a result of your findings?

Group 4 – You did X number of tests. Why did you choose those tests?

Group 5 – You only did Y number of tests. Why did you choose those tests?

You will find that some groups will only do the tetrazolium and Feulgen tests. They will indicate that they stopped there because if neither of these was positive there was little hope that the sample was alive. Other groups will do all available tests. When you ask them why they did them all, they frequently respond “because we wanted to see what we’d get and what tests we could eliminate, etc.”.

Your response as instructor to all of the students answers should be “OK”.

Then go on to the next group.

The point of doing this is to have the students discover for themselves that there might be better ways of doing the testing than the one they chose. That would be a great discovery for them to make.

You should also make it clear that their final proposal should be what they feel would be the best plan to use. They do not have to use the same plan they did for their preliminary trials. The purpose of the preliminary trials is to discover how to improve on their design.