Under the Sea

Activity 4.2.2. Screening sponge extracts for anticancer compounds

Four class days. Day one is bioprospecting and the ethics fight it starts. Day two is micropipetting and aseptic technique, practised until they are boring. Day three is the qualitative screen. Day four is the standard curve, the numbers, and your recommendation. Days two and three are the ones the WebXam actually weighs.

18 DECEMBER | WET LAB | MICROPIPETTES | ASEPTIC TECHNIQUE | LAB NOTEBOOK

READ THIS FIRST. WHAT THIS LAB CAN AND CANNOT TELL YOU

- **A colour change is not a cure.** What you are running is a screen. A screen sorts a pile of candidates into *worth another look* and *not worth another look*. It does not establish that anything treats cancer in a person.
- **Module I is qualitative. Module II is quantitative.** Module I gives you a description, positive or negative. Module II gives you a number. The number is not more true than the description. It is a different kind of claim with its own ways of being wrong.
- **Your concentrations come from a curve you built yourself.** Every number you report is read off a standard curve made from tubes you pipetted. If your standards were pipetted badly, your unknowns are wrong by exactly the same amount and nothing on your sheet will warn you.
- **Five sponges is not a study.** Real high throughput screening runs thousands of compounds through robots. You are running five by hand so that you understand what the robot is doing.
- **There is no answer key.** Which sponges come out positive, and what their concentrations are, is not printed here or anywhere you can reach. Finding out is the assignment.

Day one. Bioprospecting, and the argument about it

There are two ways to get a new therapeutic. Design one from scratch in a laboratory, or go find one that already exists in a living thing.

The second route is called **bioprospecting**, and it is older than laboratories. People drank tea made from willow bark for fever and pain long before anyone knew that willow bark contains salicylic acid, which is the basis of aspirin. Bioprospectors look hardest at organisms nobody has studied much, on the reasoning that the well-studied ones have already given up their secrets.

CONDITION	THERAPEUTIC	WHERE IT CAME FROM
Lung, ovarian and breast cancer	Taxol	Bark of the pacific yew tree. A functionally synthetic version is now made in the lab, which means the trees are no longer harvested for it.
Alzheimer's disease	Galantamine	The snowdrop plant. Used to support memory in a disease that progressively destroys memory and brain function.
Acute coronary syndrome	Aggrastat	Venom of the saw-scaled viper. It is an anticoagulant, meaning it breaks up clots, and acute coronary syndrome is a sudden drop in blood flow to the heart.
Obesity	An adenylate cyclase compound	Miers, a plant found in East Asia. Shown to help control weight.

WHY BIODIVERSITY IS A SUPPLY CHAIN, NOT A DECORATION

Every one of those four came from a species somebody had to still be able to find. The greater the variety of life, the greater the genetic variation between species, and the more chemistry there is that nobody has looked at yet. A species that goes extinct takes its chemistry with it, including the parts nobody got round to testing. That is the argument you will be asked to make, and it is worth being able to make it in your own words.

THE ETHICS QUESTION, STATED HONESTLY WITH NO ANSWER ATTACHED

You find a bioactive compound in a sponge. It might help people with cancer. It might also be worth a great deal of money. Companies protect discoveries like this by filing **patents** on biological resources. A patent limits who else may investigate or experiment on that resource.

So: should a company be able to patent a living organism, or a compound that organism makes and that the company did not invent? If yes, what does the country the sponge came from get? If no, who pays for the decade and the money it takes to turn a hit in a plate into a medicine a person can take?

Both answers have real costs. You are expected to pick one and defend it against the strongest version of the other side, not against the weakest.

MAPPING AND PRIVACY, IF YOUR CLASS ALSO RAN ACTIVITY 4.2.1

Geographic information systems are used in public health to track disease spread by tracking where infected people are. That is useful and it maps individual people. The same tension shows up here that shows up in the patent question: a genuine benefit and a genuine cost, and no option that avoids both. HIPAA is the rule set that governs what may be done with identifiable health information in the United States.

Day two. Micropipetting and aseptic technique

This is the day that shows up on the WebXam. The 072110 exam weighs handling, preparation, storage and disposal more heavily than anything else, and a micropipette is the tool that whole domain is built around. Practise until it is boring.

A micropipette measures volumes in **microlitres**. One microlitre is one thousandth of a millilitre and one millionth of a litre. Your variable micropipette covers 10 to 100 microlitres. That range is small enough that mistakes are invisible: a pipette that delivers 85 microlitres when it says 100 looks exactly like a pipette that is working.

1. **Check the range before you set the volume.** Never dial a micropipette past either end of its printed range. Forcing it past the top of the range damages the mechanism and the damage is not visible from outside.

   Done when: the number in the window is inside the range printed on the barrel.

2. **Fit a fresh tip.** Press the shaft straight down into the tip and give it a small firm push. Do not touch the tip with your fingers. Do not reuse a tip between samples, ever.

   Done when: the tip is on straight and seated, and no part of it has been touched by a hand.

3. **Press to the first stop to draw up.** The plunger has two stops. You will feel the first one as a change in resistance. Press only to the first stop, put the tip into the liquid, then release the plunger slowly and smoothly.

   Done when: no bubble is visible in the tip. A bubble means the volume is wrong.

4. **Hold the pipette upright while it holds liquid.** Tilting it lets liquid run back into the shaft, which contaminates the pipette and changes the volume.

5. **Press to the second stop to dispense.** Touch the tip to the wall or the bottom of the receiving tube, press smoothly past the first stop to the second, and hold it down while you withdraw the tip. Release the plunger only after the tip is out of the liquid.

   Done when: the tube received the full volume and nothing ran back up the tip.

6. **Eject the tip into the waste container.** Use the ejector button, not your fingers.

   Done when: the tip is in the container and your hand has not been near it.

7. Practise the whole cycle at three different volumes until you can do it without reading this list.

   Done when: you can run the cycle correctly twice in a row while talking to someone.

ASEPTIC TECHNIQUE, AND WHY IT MATTERS EVEN WHEN NOTHING IS ALIVE

Aseptic technique is the set of habits that keeps unwanted material out of your samples and keeps your samples out of you. It is not only about infection. In a screening assay, a stray microlitre carried from tube 3 into tube 4 on a reused tip produces a result in tube 4 that is not about tube 4. You will read that result, believe it, and report it.

- One fresh tip per sample. No exceptions, no matter how small the volume looked.
- Keep tubes closed except in the moment you are pipetting into or out of them.
- Work over the bench, not over your lap or the floor. Do not lean across an open tube.
- Label before you fill. A rack of identical clear tubes is unrecoverable ten seconds after you lose track of it.
- Hands washed at the start and at the end, whether or not you wore gloves.

Activity 4.2.2 asks you to explain why aseptic technique matters in this lab. The answer worth writing is about your data, not only about safety.

Safety

STANDING RULES FOR BOTH MODULES

- **Splash goggles on before any container is opened and until the bench is wiped.**
- Long hair tied back. No food, no drink, nothing near your mouth. Do not smell any sample.
- **Hot water bath or hot plate.** The bath and anything that has been in it are hot and do not look hot. Use tongs or a rack to move tubes. Never reach across a hot plate. Know where the switch is before you turn it on.
- Tubes warmed in a bath build pressure. Open them slowly and pointed away from every face at the bench, including your own.
- Report every spill and every splash, including small ones.
- Used tips and used tubes go in the container Mr. Mendoza sets out. Not the regular bin, not the sink. Ask before you pour any liquid down a drain.
- Wipe the bench and wash your hands at the end of every module.

Day three. Module I, the qualitative screen

All of your sponges have already been through high throughput screening, or **HTS**, which is the robotic process real laboratories use to test enormous numbers of compounds quickly. HTS flagged five sponges as producing compounds that appear active against cancer cells. Your job is to screen those five by hand.

This module is **qualitative**, meaning the data you collect is descriptive. Each sponge comes out positive or negative. The readout is colour: samples producing the bioactive compound turn deep purple, and samples producing none or very little stay clear.

1. Collect the Laboratory Procedure and the Student Worksheet. Read the Module I procedure end to end before you touch anything.

   Done when: you can name the last step before you start the first.

2. Write in your notebook, in your own words, why aseptic technique matters in this particular experiment. Do this before you run it, so the reason is a prediction rather than an excuse.

   Done when: a written reason exists and it mentions your data, not only your safety.

3. Label your tubes before you fill anything. Sponge number on every tube.

4. Extract the compounds and run the screen following the procedure. One fresh tip per sample.

   Done when: five samples were handled with five tips.

5. Record your observations. Describe the colour you actually see, not the colour you expected.

   Done when: every sponge from 1 to 5 has a written observation, including the ones you found ambiguous.

6. Clean up. Tips and tubes to the waste container. Wash your hands.

RECREATE THIS TABLE IN YOUR NOTEBOOK. FILL THE THIRD COLUMN ON DAY FOUR.

SPONGE NUMBER	MODULE I. RESULT OF QUALITATIVE SCREEN	MODULE II. CONCENTRATION OF BIOACTIVE COMPOUND
1		
2		
3		
4		
5		

THE TWO QUESTIONS MODULE I ASKS, AND A WARNING ABOUT THE SECOND ONE

First: which sponges were producing bioactive compounds, and how do you know? The second half of that question is the graded half. *Because it turned purple* is a start. Name what purple means in this assay and what a clear tube rules out.

Second: did all five sponges produce anticancer compounds? If not, how would you explain the disagreement with the HTS study, which flagged all five?

That second question is not a trick and it is not an error in the material. A robotic screen and a hand screen are two different measurements of two different things, run under different conditions, with different sensitivity. There are several defensible explanations for a disagreement between them and this handout is not going to hand you one. Work out what your own assay could and could not have detected, and argue from that.

Day four. Module II, putting a number on it

Module II is **quantitative**. You compare your samples against a set of tubes whose concentrations you already know, and read your unknowns off the resulting curve.

A **standard curve** is how you turn an observation into a number. You prepare a series of tubes at known concentrations, treat every one of them exactly the way you treat your unknowns, and plot the result. Then any unknown that got the same treatment can be located on that curve.

THE STANDARD SERIES. RECORD THESE IN YOUR NOTEBOOK BEFORE YOU BEGIN.

TUBE	A	B	C	D	E	F	G
Standard solution concentration (mg/mL)	10	5	2.5	1.25	0.63	0.31	0.16

LOOK AT THE SPACING OF THAT SERIES BEFORE YOU PLOT IT

Each tube is roughly half the one before it. That is a halving series, not an evenly spaced one. Plotting halving concentrations on evenly spaced axis marks gives you a curve that bends sharply, and the bend is where reading it gets least reliable. Notice where your unknowns land relative to that bend, because an unknown sitting on the flat part of a curve carries far more uncertainty than one sitting on the steep part, and no number on your sheet tells you that.

1. Read the Module II procedure end to end before you begin.
2. Record the concentration in each standard tube A to G in your notebook.

   Done when: seven concentrations are written down with their units.
3. Run Module II following the procedure. Same pipetting discipline as day three. One fresh tip per tube, every time.
4. Build your standard curve from the standards. Plot concentration against the result you measured.

   Done when: a plotted curve exists with both axes labelled and both units written on.
5. Read each of your unknown samples off the curve and add the numbers to the third column of your results table.

   Done when: every sponge has either a concentration or a written reason why it could not be read.
6. Write your recommendation. Which sponge or sponges should the Drug Design Lab continue to study, and why?
7. Clean up as directed. Wash your hands.

HOW TO READ AN UNKNOWN OFF A CURVE

Find your measured result on the horizontal axis. Move straight up until you meet the curve. From that point move straight across to the vertical axis. Where you land is the concentration. Read it as a range, not a point, if your result sits between two standards.

PRACTICE EXAMPLE PRACTICE-CURVE-1. INVENTED DATA, INVENTED COMPOUND, NOT THIS LAB

> **Setup.** A practice standard curve for an invented compound called PC-1. Seven standards were prepared at 20, 10, 5, 2.5, 1.25, 0.63 and 0.31 micrograms per millilitre and every tube was treated identically. Colour was scored on the same scale as the unknowns.
>
> **Reading unknown P.** Unknown P scored between the 5 and the 2.5 standard, closer to 5. Moving up from that score to the curve and across to the axis gives roughly 4 micrograms per millilitre. Reported as *about 4, between 2.5 and 5*, because the reading sits between two standards and the curve is steep there.
>
> **Reading unknown Q.** Unknown Q scored darker than the 20 standard. That is off the top of the curve. The honest report is *greater than 20, cannot be quantified with this standard series*. It is not 20, and it is not 25. Extending a curve past its highest standard is guessing with a ruler. To get a number, the sample would have to be diluted and rerun.
>
> **Reading unknown R.** Unknown R was indistinguishable from the blank. Reported as *below the lowest standard, 0.31, not detected by this assay*. Not detected is not the same as zero. It means this assay at this sensitivity did not see it.

THREE WAYS TO REPORT A NUMBER HONESTLY, ALL SHOWN ABOVE

- **Between two standards.** Give the estimate and the bracket it sits in.
- **Off the top.** Say greater than your highest standard and say it cannot be quantified. Do not extend the curve.
- **Below the bottom.** Say not detected by this assay, not zero.

Every one of those three is a complete, professional answer. A made up number that looks precise is worse than any of them.

YOUR RECOMMENDATION

Write a claim, evidence, reasoning response. Which sponge or sponges should the Drug Design Lab keep working on?

- **Claim.** Name the sponge or sponges. One sentence.
- **Evidence.** Your Module I results and your Module II concentrations, both. Say where each number came from and how confident the reading was.
- **Reasoning.** Why does that evidence support that choice? A high concentration is one reason to keep going, and it is not the only one. Consider what your Module I and Module II results say together, and what you would do about any sponge where the two disagree.
- **What you are not sure about.** Name at least one thing. Where your curve was hardest to read, where your pipetting was rushed, where a tube looked ambiguous and you had to make a call.

What happens after a hit

Finding a compound in a plate is the beginning of a very long road, and knowing the shape of that road is part of the point of this activity.

STAGE	WHAT HAPPENS	SCALE
Screening	Compounds are tested for activity. Most fail here.	Thousands of candidates.
Pre-clinical studies	Promising compounds are tested in animals and in computer models for safety and effect.	Of 5,000 to 10,000 candidates evaluated, roughly five reach the end of pre-clinical testing.
Clinical trials	A series of studies in humans.	Years.
FDA review	The Food and Drug Administration, a federal agency inside the Department of Health and Human Services, decides whether the benefit outweighs the side effects.	The whole process can take more than a decade and cost up to a billion dollars. Prozac spent fifteen years and seven months in development before approval.

> **HOLD THOSE TWO NUMBERS NEXT TO EACH OTHER**
>
> Five survivors out of ten thousand. More than a decade. That is the arithmetic behind the patent argument you had on day one, and it is also the arithmetic behind the question of whether the process should ever be sped up. Both sides of that argument are standing on this same table.

Where people get stuck

Reusing a pipette tip because the volume was small.

Carryover from one tube to the next produces a result in the second tube that belongs to the first. You will never see it and you will report it as data. One tip per sample.

Filling tubes before labelling them.

Five identical clear tubes in a rack are unrecoverable the moment you lose the order. Label first, every time.

Bubbles in the tip.

A bubble means you did not draw the volume you dialled. Release the plunger slowly and evenly, and look at the tip before you move it.

Reading an unknown off the flat end of the curve and reporting it to two decimal places.

Where the curve is flat, a tiny difference in colour is a large difference in concentration. Report a range, and say the curve was flat there.

Extending the curve past the highest standard.

A sample darker than your top standard has no number. Say so. Diluting and rerunning is the real fix, and saying that is a complete answer.

Treating not detected as zero.

It means your assay at your sensitivity did not see it. A more sensitive method might. Those are different claims and only one of them is supported.

Deciding what colour a tube is after seeing what the group next to you got.

Score your own tubes before you compare, and write the score down. Then compare, and if you disagree, that disagreement is worth a line in your notebook.

Answering the HTS disagreement question with *the HTS was wrong* and stopping.

That is a claim without reasoning. Say what your assay could detect, what it could not, and what conditions differed between the two. Then a disagreement becomes evidence rather than a shrug.

Skipping the ethics work because it is not the lab.

It is graded, it is on the exam register for this unit, and it is the part you will still be arguing about in ten years.

Words this activity uses

Therapeutic

A treatment, therapy or drug.

Novel

Brand new. A novel therapeutic is one designed from scratch rather than found in nature.

Bioprospecting

Searching living things for useful bioactive compounds. Organisms nobody has studied closely are the usual targets.

Bioactive compound

A chemical made by a living thing that has an effect on another living thing.

Biodiversity

The variety of life. Greater variety means greater genetic variation between species, which means more untested chemistry.

High throughput screening

HTS. Using robotics and computers to test very large numbers of compounds quickly, because doing it by hand would take years.

Qualitative data

Descriptive. Positive or negative, purple or clear.

Quantitative data

Numerical. A concentration in milligrams per millilitre.

Standard curve

A plot built from samples of known concentration, treated identically to your unknowns, used to convert a measurement on an unknown into a concentration.

Concentration

How much of a substance sits in a given volume.

Microlitre

One millionth of a litre, one thousandth of a millilitre. The unit a micropipette measures in.

Aseptic technique

The working habits that keep unwanted material out of your samples and your samples out of you.

Carryover

Material moved from one sample into the next, usually on a reused tip.

Patent

A legal protection over a discovery. Patents on biological resources limit who else may investigate or experiment on them.

Pre-clinical study

Testing a candidate compound in animals and computer models before humans.

Clinical trial

A series of studies testing a candidate compound in humans.

FDA

The Food and Drug Administration, a federal agency of the United States Department of Health and Human Services, which decides whether a drug may be marketed.

Anticoagulant

A substance that breaks up or prevents blood clots.

> **CAREERS THIS LAB TOUCHES**
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> **Marine biologist.** Dr. Kim Ritchie splits her time between the field and the laboratory, bioprospecting for new antibiotics to fight bacterial infections. Activity 4.2.2 links a Shark Expedition video of her work.
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> **Marine biotechnologist.** Tests and evaluates ocean life looking for useful products and therapeutics. This is the role you are standing in for across Activities 4.2.1 and 4.2.2.