

Name the Pathogen

The evidence circuit that closes the outbreak investigation. Five tests, five classes of agent, one name at the end.

COURSE Medical Interventions	DATE Thursday, September 17, 2026	BLOCK 80 minutes
STATIONS 5 mini labs	WET LAB SEC 03 only	TOOL NCBI BLAST, in SEC 06

YOUR CHARGE TODAY

By the end of this block you will name the class of agent causing this outbreak, then name the organism itself, and you will be able to say which piece of evidence earned each of those two claims.

YOU TURN IN

- **One completed packet**, every green block filled in.
- **Two graphs**, the dilution series and the gel standard curve.
- **One identification paragraph** in SEC 07 with your three BLAST numbers in it.
- **Extra credit, at home:** the physician's treatment plan, a separate sheet.

Time: 80 minutes, in class. The wet lab is timed. Everything else is at your own pace.

GREEN RULE You write here. Nothing else on the page is yours to fill in.	GOLD GROUND Given to you. Do not re-derive it. Controls and reference values live here.	GREY GROUND Instructions from Mr. Mendoza. Read before you start the step.	RED GROUND Safety. Read it before you touch any material.
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WHY THIS MATTERS

Infectious disease still kills millions of people a year worldwide, and the single largest reason a treatable infection becomes a fatal one is time lost naming it. A doctor who does not know the class of agent is choosing a drug by guess, and a guess against the wrong class does nothing at all.

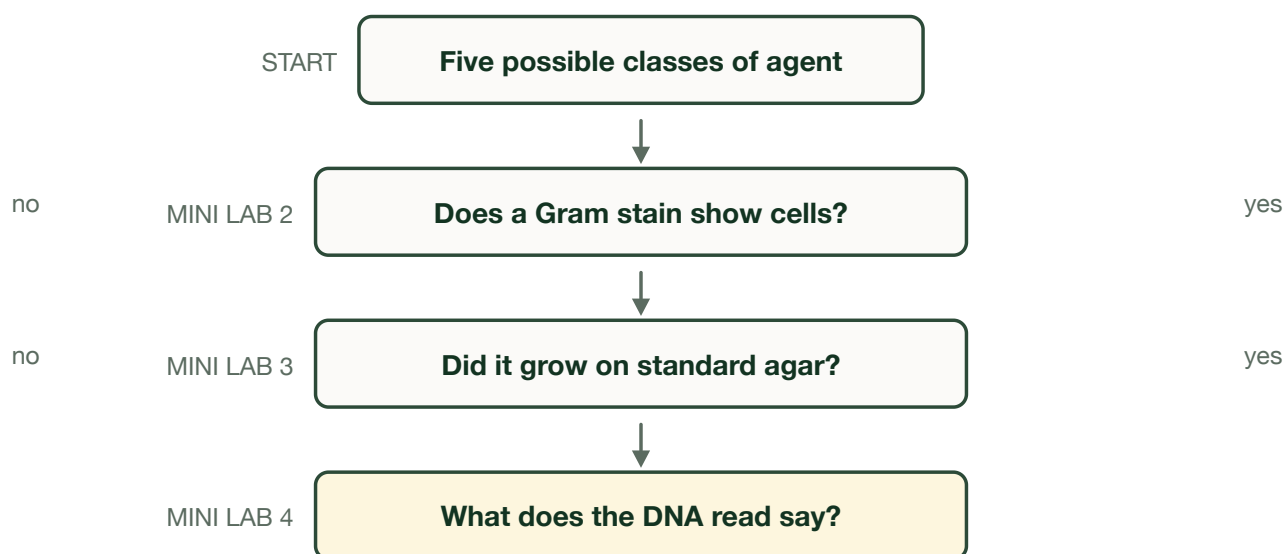
READ THIS

An antibiotic does nothing to a virus. An antifungal does nothing to a bacterium. Before anyone can choose a treatment, somebody has to answer a narrowing question: **what kind of thing is this?**

There are five classes on the table today. Each mini lab removes some of them. By SEC 06 you should be down to one class, and BLAST gives you the organism inside it.

THE DECISION YOU ARE MAKING TODAY, AND THE ORDER YOU MAKE IT IN

Each test below removes possibilities. None of them, on its own, names an organism.



The order of the work. It does not supply the answer at any branch; each mini lab produces one of those answers.

CONTROL GROUP · ALREADY SET UP FOR YOU

The reference table of agent characteristics is in the index at the back of this packet. It is given to you, it is not something you derive today, and its sources are printed with it. Turn to it whenever a mini lab asks you to rule a class in or out.

YOUR WORK · YOU COMPLETE THIS

Before any evidence: write which class you would guess, and why. You will check this against your own result in SEC 07.

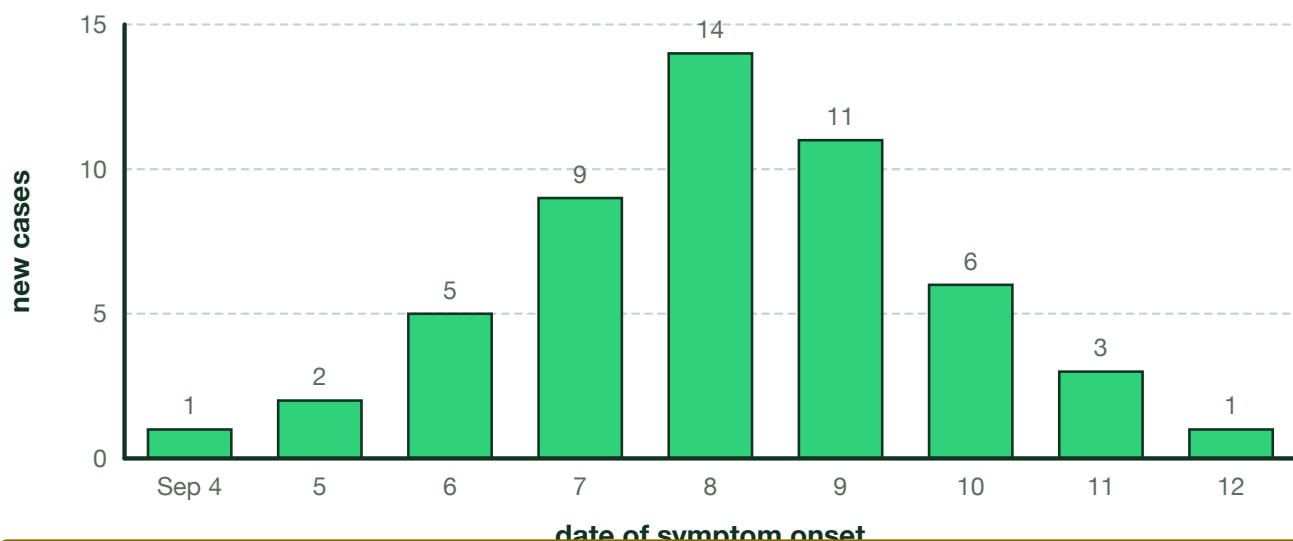


WHY THIS MATTERS

Epidemiology is the part of medicine that works on populations rather than one patient, and the shape of an outbreak curve has redirected real investigations. One sharp peak says everybody met the same thing once. A rolling series of peaks says people are giving it to each other, and those two findings call for completely different responses.

READ THIS

You have two pieces of population evidence: the patient record in your evidence portfolio, and the outbreak curve below. Neither names an organism. Both narrow the field.

EXHIBIT MI-2: new skin infections by date of symptom onset

One sharp peak about one incubation period after a shared event points to a single common exposure. Several successive peaks would point to spread from person to person. Read the shape, not the height.

The outbreak curve. Case counts are printed because they are the record you were given. It does not supply the shape's meaning or the exposure date; you write both.

DO THIS

- ➊ Read the patient record in your evidence portfolio, section 1. List the three findings you think matter most.
- ➋ Look at the curve. Decide whether it shows one shared exposure or spread from person to person.
- ➌ Using the onset peak and a typical incubation period, estimate the day the exposure happened.
- ➍ Open the agent characteristics table in the index. Using onset speed alone, write which classes stay possible.

MINI LAB 1 • NARROW BY POPULATION EVIDENCE

Agent class	Still possible after the curve? yes / no	The evidence that decided it
Bacteria		
Viruses		
Fungi		
Protozoa		
Parasitic worms		

YOUR WORK · YOU COMPLETE THIS

Does this curve show one shared exposure or person to person spread? Give the feature of the shape that told you.

Which classes did you rule out on onset speed alone, and which stayed in?

WHY THIS MATTERS

Every blood test that reports a number rather than a yes or no depends on a standard curve, and a standard curve is built from a dilution series. The series you make in the next twenty minutes is the one tomorrow's ELISA is read against, so a careless tube today becomes a wrong concentration tomorrow.

READ THIS

This is the only wet lab today, and all three of my classes run it, so the bench is set the same way in every period.

A **serial dilution** is the same dilution repeated. Each tube takes 1.0 mL from the tube before it into 9.0 mL of water, so each tube is one tenth as concentrated as the one before. Five steps takes you from the stock to one hundred thousandth of it.

You are using hydrochloric acid and the indicator phenolphthalein. The indicator is colourless in acid and pink above pH 8.2, so counting the drops of base needed to turn each tube pink measures how much acid is in it. That drop count against dilution step is your standard curve.

SAFETY · READ BEFORE YOU TOUCH ANYTHING

- **Goggles on before the rack is in front of you.** Aprons on. Long hair tied back.
- 0.1 M hydrochloric acid and 0.1 M sodium hydroxide are both dilute, and both still burn eyes.
- The phenolphthalein is made up in alcohol and is flammable. No flame at this station.
- Anything on skin goes under the tap for a full minute first, then you tell me.
- Everything is rinsed into the labelled waste carboy, not the sink.

CONTROL GROUP · ALREADY SET UP FOR YOU

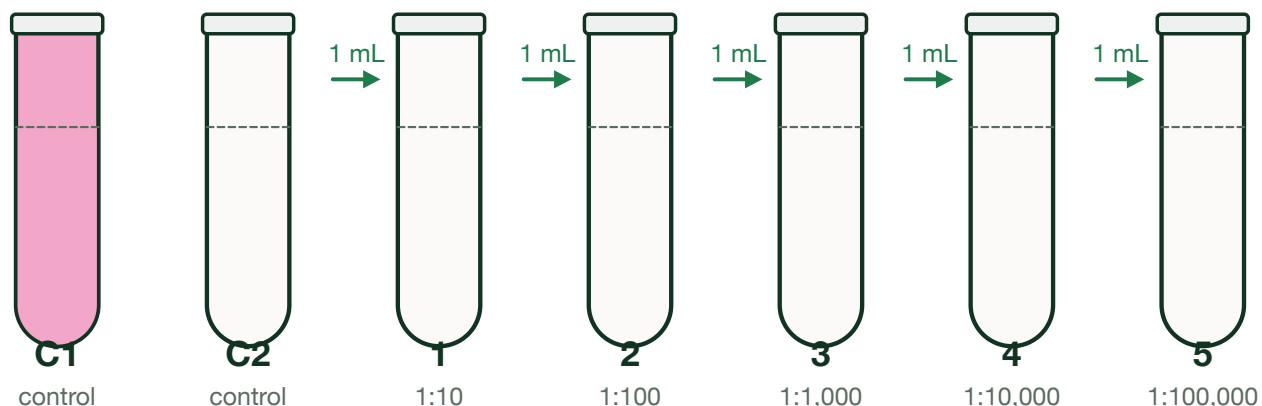
Two control tubes are already made up in your rack.

- **C1, base plus indicator, no acid.** Pink all block. It proves the indicator works.
- **C2, distilled water plus indicator only.** Colourless all block. It proves the glassware is clean.

Check both before tube 1 and again at the end, and record both checks. If C1 is not pink, stop.

SET-UP: the rack as it will be waiting at your station

Each tube already holds 9.0 mL of distilled water. You add 1.0 mL from the tube to its left, mix, and move on.



C1: base plus indicator, no acid added.
Stays pink. Shows the indicator works.

C2: distilled water plus indicator only.
Stays colourless. Shows water alone is not the cause.

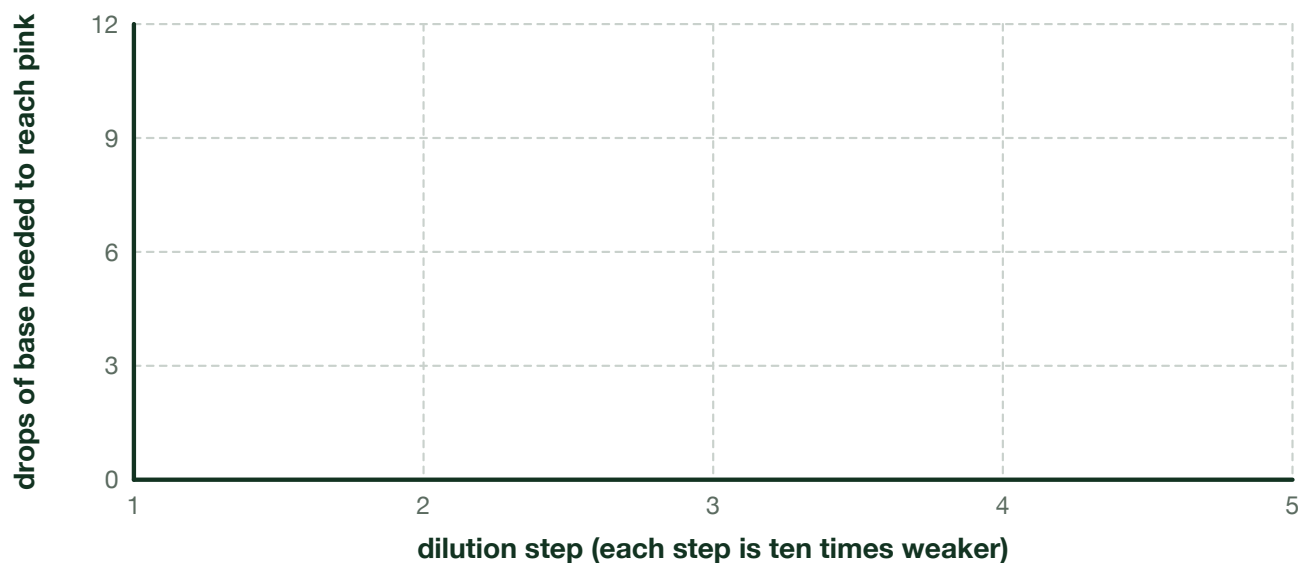
The rack as it will be waiting. The controls are drawn with their colours because a control is given. It does not supply any result for tubes 1 to 5.

DO THIS

- ➊ Check C1 and C2 and record both. If either is wrong, raise your hand before you touch anything else.
- ➋ Tube 1 holds 9.0 mL of water. Add 1.0 mL of stock acid. Cap and invert three times.
- ➌ Carry 1.0 mL from tube 1 to tube 2, invert, and repeat down to tube 5. New tip every transfer.
- ➍ Add two drops of phenolphthalein to tubes 1 through 5.
- ➎ Add 0.1 M sodium hydroxide to tube 1 one drop at a time, swirling, until pink holds for five seconds. Record the drops.
- ➏ Repeat for tubes 2 to 5. Re-check C1 and C2. Then plot drops against dilution step.

MINI LAB 2 · THE DILUTION SERIES AND TOMORROW'S STANDARD CURVE

Tube	Dilution factor	Concentration mol/L	Drops to reach pink drops
C1	('control',)	('0.1 M base',)	
C2	('control',)	('water only',)	
1	('1:10',)	('0.010',)	
2	('1:100',)	('0.0010',)	
3	('1:1,000',)	('0.00010',)	
4	('1:10,000',)	('0.000010',)	
5	('1:100,000',)	('0.0000010',)	



Keep this graph. Tomorrow you read the model ELISA wells against this exact line.

Your standard curve. It does not supply any point or the line through them.

YOUR WORK · YOU COMPLETE THIS

Each step is ten times weaker. Describe what happened to the drop count at each step.

Which was the last tube where you could still detect the acid? That is your detection limit.

Why can a standard curve tell you the concentration of an unknown sample? Answer in one sentence.

WHY THIS MATTERS

The Gram stain is over 140 years old, costs almost nothing, and is still run within minutes of a serious infection arriving in a hospital, because it splits the bacterial world in two and that split changes the first antibiotic given. Few tests in medicine buy that much for that little.

READ THIS

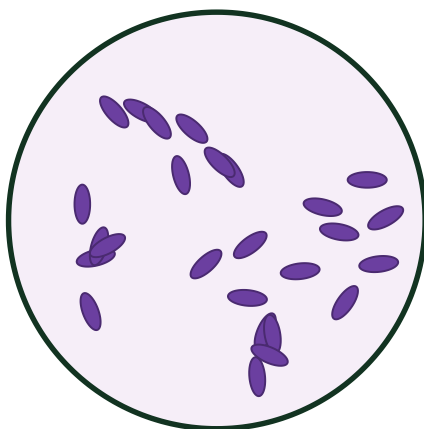
A Gram stain answers two questions at once: **is anything there**, and **what shape and stain type is it**. Purple means the cell held the first stain (Gram positive). Pink means it did not and took the counterstain instead (Gram negative). Round cells are cocci. Rod-shaped cells are bacilli.

The culture answers a different question: **will it grow on ordinary agar**. That single result separates bacteria and fungi from viruses, protozoa and worms.

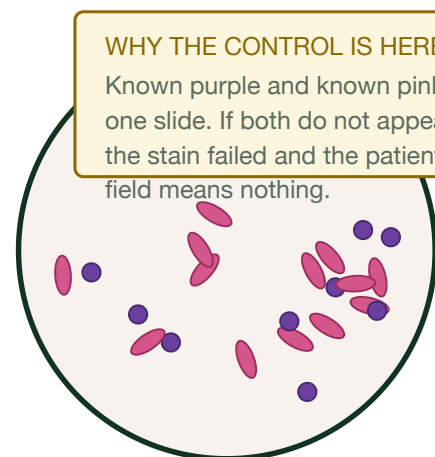
CONTROL GROUP • ALREADY SET UP FOR YOU

Two controls, each ruling out a different failure.

- **The stain control slide** carries known Gram positive cocci and known Gram negative rods. If both colours do not appear, the stain failed and the patient slide is meaningless.
- **The sterile control plate** was handled exactly like the patient plate but never inoculated. If anything grows on it, your patient colonies could have come from the room instead of the patient.

EXHIBIT MI-3: Gram stain, patient isolate and the stain control**PATIENT ISOLATE**

1000x, oil immersion

**STAIN CONTROL, known organisms**

1000x, oil immersion

WHY THE CONTROL IS HERE

Known purple and known pink on one slide. If both do not appear, the stain failed and the patient field means nothing.

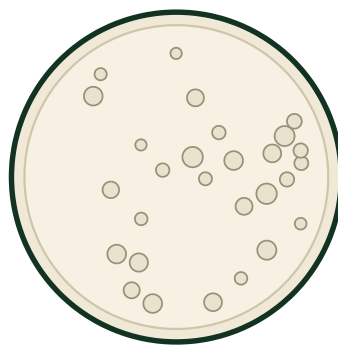
The Gram stain and its control. It does not supply the Gram result or the cell shape in words; you record those.

EXHIBIT MI-4: culture at 24 hours, 37 degrees Celsius



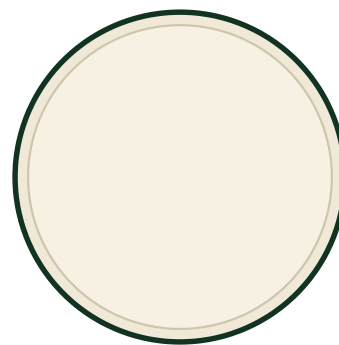
PATIENT SAMPLE

count and describe in your table



POSITIVE CONTROL

growth, as it should be



STERILE CONTROL

no growth, as it should be

The 24 hour culture. It does not supply a colony count, a description or a conclusion.

DO THIS

- 1 Check the stain control. Record whether both known colours appeared.
- 2 Check the sterile plate. Record whether it stayed clear.
- 3 Record the Gram reaction of the patient isolate: purple or pink.
- 4 Record the cell shape: round (coccus) or rod (bacillus).
- 5 Record whether the patient sample grew on standard agar.
- 6 Return to the agent characteristics table and cross out every class these three results have ruled out.

MINI LAB 3 · NARROW BY STAIN AND CULTURE

Observation	Your result	What it rules out
Stain control passed?		('if no, stop',)
Sterile plate stayed clear?		('if no, stop',)
Gram reaction of the isolate		
Cell shape		
Grew on standard agar?		

YOUR WORK · YOU COMPLETE THIS

After the stain and the culture, which single class of agent is still standing? Name the two results that removed the others.

A classmate says the stain proves which species it is. Write the one sentence you would say back.



WHY THIS MATTERS

Running a patient sample against a known ladder is how a laboratory turns an invisible molecule into a measurement anyone else can check. The same method sizes a virus fragment, confirms a bacterial gene, and reads a DNA profile in a criminal case. One idea, used three ways.

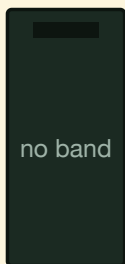
READ THIS

The laboratory ran PCR on the isolate using primers for a gene region that differs between bacterial species, then ran the products on a gel. Short fragments travel further than long ones, so the distance a band travelled tells you its size once you have a ladder to read against.

This gel narrows the candidates. It does not name one. That is the next mini lab.

CONTROL GROUP • ALREADY SET UP FOR YOU

The same two control lanes as any run: a no-template lane that must stay empty, and a positive control that must show its band. Check both before reading anything else.

THE TWO CONTROL LANES, AND WHAT EACH ONE RULES OUT**NO-TEMPLATE CONTROL**

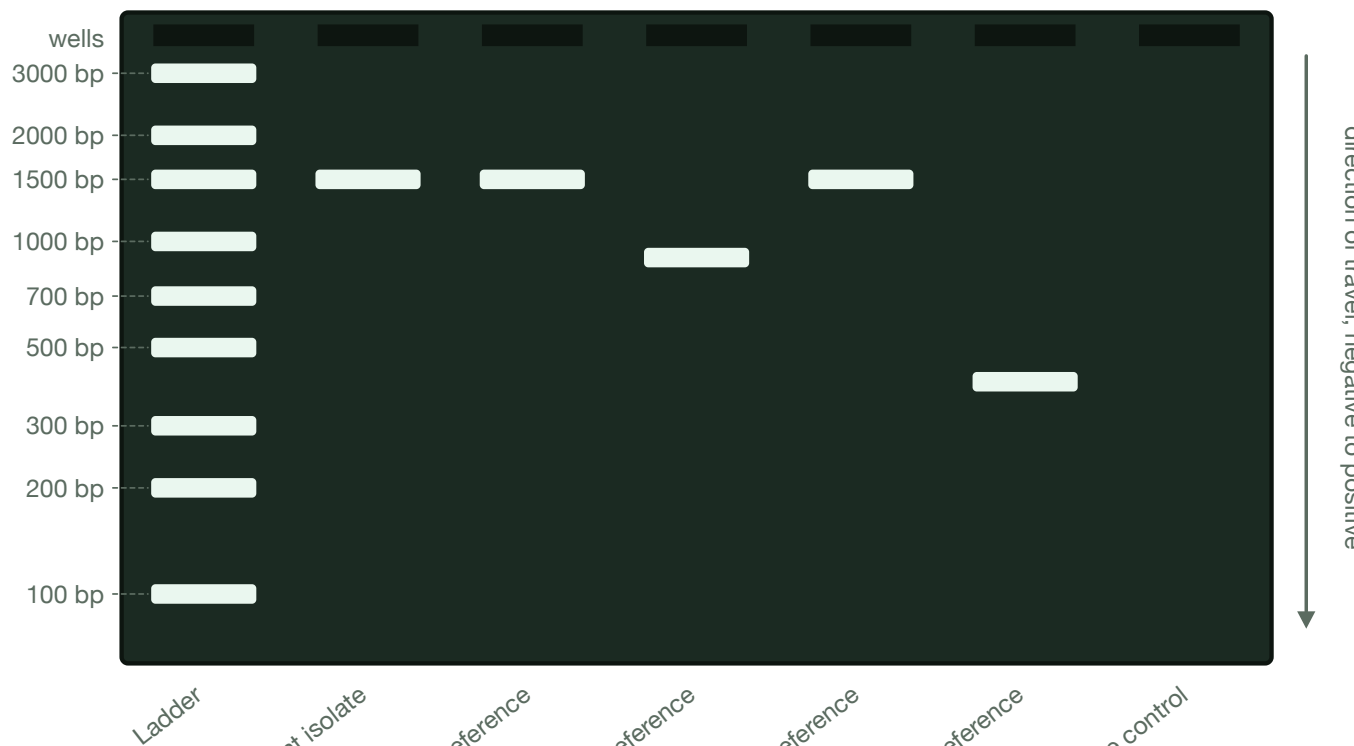
Everything except DNA. If a band appears here, the reagents are contaminated and every lane is void.

**POSITIVE CONTROL**

DNA known to amplify at 800 bp. If nothing appears here, the run has failed and an empty sample lane means nothing.

The two control lanes. It does not supply whether the controls on your own gel passed.

EXHIBIT MI-5: PCR product from the patient isolate, against four candidate reference

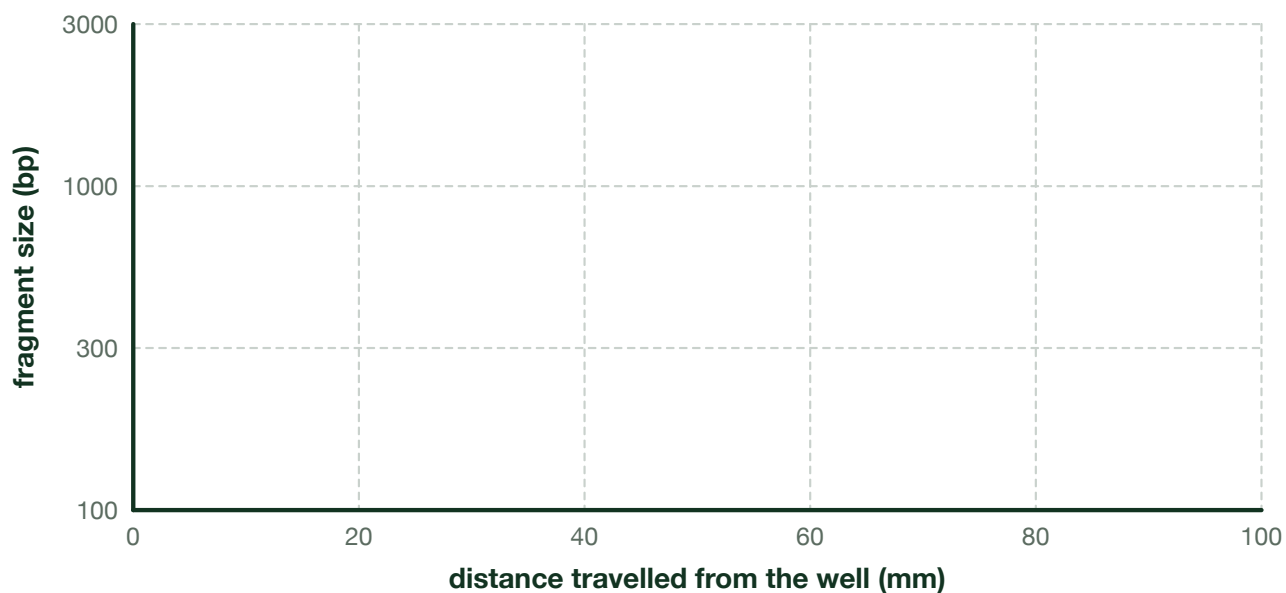


Lane 1 is the ladder. Its fragment sizes are printed because a ladder is the instrument you measure against, not an answer.

The evidence gel. Ladder sizes are printed because the ladder is the instrument. It does not supply the size of the patient band or which candidates remain.

DO THIS

- 1 Check both control lanes and record the result.
- 2 Measure from the bottom of each well to the centre of each band in millimetres.
- 3 Plot the nine ladder bands on the graph and draw one straight line through them.
- 4 Read the patient band off your line and record its size in base pairs.
- 5 Record which candidate references produced a band of the same size.



Plot the ladder first. Every unknown you report has to be read off this line, not guessed from the picture.

Build the standard curve here. It does not supply a ladder point or the line.

MINI LAB 4 · SIZE THE PCR PRODUCT

Lane	Distance travelled mm	Size from your curve bp	Same size as the patient? yes / no
Patient isolate			
Candidate A			
Candidate B			
Candidate C			
Candidate D			

YOUR WORK · YOU COMPLETE THIS

How many candidates still match the patient band, and which are they?

You have more than one candidate left with the same band size. Write the one sentence that says what this gel can and cannot establish.

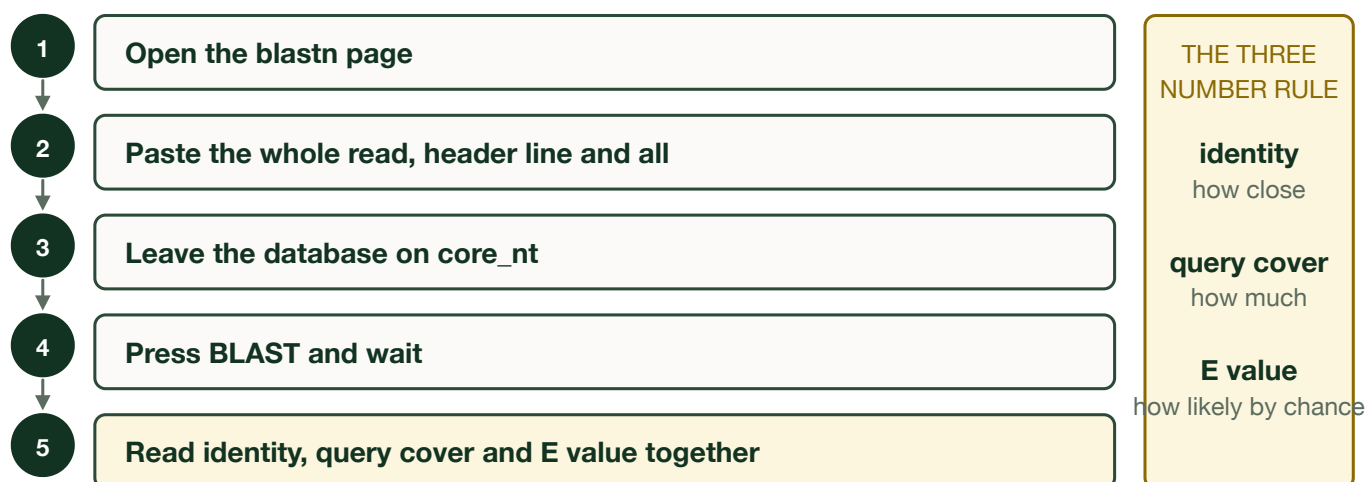
WHY THIS MATTERS

Sequencing turned identification from a week of culturing into an afternoon, and public databases mean a school in Cleveland runs the same search a reference laboratory runs. The read below is a real 16S sequence and the tool is the real tool. Nobody in this room, including me, is going to tell you the answer, because the whole point is that the evidence tells you.

READ THIS

The 16S rRNA gene is carried by every bacterium. It is similar within a species and different between species, which is what makes it the standard gene for naming a bacterium from its DNA.

Below is one 500 base read from the patient isolate. **The name of this organism appears nowhere in this packet, nowhere in your portfolio, and nowhere on the portal.** You are going to get it from BLAST.

THE ONLY STEP THAT CAN NAME THE ORGANISM

The five steps and the three numbers. It does not supply a hit, a number or a name.

CONTROL GROUP • ALREADY SET UP FOR YOU

The three number rule, which you already used on Monday. Read all three together before you name anything.

- **Percent identity:** of the bases that lined up, how many matched exactly.
- **Query cover:** how much of your read took part in the alignment. A brilliant match on ten percent of the read is not a brilliant match.
- **E value:** how many matches this good you would expect by chance. Smaller is stronger, and 0.0 means too small to display.

EXHIBIT MI-6 · THE ISOLATE READ

Your isolate read. Copy the whole thing, including the line that starts with the greater-than sign.

```
>S2_practice_isolate 16S rRNA gene, one read of 500 bases
GGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACGGACGAGAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACACG
TGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTG
TCACTTATAGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGG
AACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTT
CGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAAGTGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTA
```

DO THIS

- 1 Open the blastn page. The link is on today's portal page and in the index.
- 2 Paste the entire read into the query box.
- 3 Leave the database set to core_nt and the program on highly similar sequences (megablast).
- 4 Run the search. While it runs, write your prediction in the green block.
- 5 When the table appears, record the top hit and the runner-up in the table below.
- 6 Apply the three number rule and write your identification sentence.

MINI LAB 5 · YOUR BLAST RECORD

	Scientific name	Accession	Identity %	Query cover %	E value
Top hit					
Runner-up, different species					

YOUR WORK · YOU COMPLETE THIS

Your prediction, written before the results appeared:

Your identification sentence. Name the organism and cite all three numbers inside the sentence.

The claim ceiling: a BLAST match names the organism your read most resembles in the database. Write what that does not establish about this patient.

WHY THIS MATTERS

Naming an organism is only useful if it changes what happens to the patient, and it does. The same infection treated with the right drug and the wrong drug produces two different outcomes, and for at least one organism on the table below the reflex answer, an antibiotic, is the wrong answer.

READ THIS

Turn to the **treatment and prognosis table** in the index. Find your organism, or the closest candidates if your three numbers did not settle it.

Read the whole row. The treatment column and the prognosis column are different questions, and for two organisms in that table the prognosis depends on a laboratory result that has not been run yet.

YOUR WORK · YOU COMPLETE THIS

The organism your evidence supports, and how confident you are in one word:

The treatment its row gives, copied exactly:

One thing about the prognosis that depends on a test nobody has run yet. Read the last row of the patient record before you answer:

Go back to SEC 01 and read your first guess. Was it right? Write which piece of evidence would have changed your mind earliest.

CONTROL GROUP · ALREADY SET UP FOR YOU

Extra credit, at home, on the separate sheet. Write the treatment plan you would give as the physician on this case: the diagnosis, the drug and why, what you would monitor, what would tell you that you were wrong, and what you would tell the patient in plain words. It is optional and it is graded on reasoning, not on getting the same answer as anybody else.

Table 1: characteristics of the five classes of infectious agent

Class	What it is	Size	Seen on a Gram stain?	Grows on standard agar?	What it responds to	Typical onset	Example illnesses
Bacteria	One cell, no nucleus (prokaryote)	0.5 to 5 micrometres	Yes, Gram stain shows them	Yes, on standard agar	Antibiotics, chosen by susceptibility	Hours to a few days	Strep throat, listeriosis, tuberculosis
Viruses	Genetic material in a protein coat, not a cell	20 to 300 nanometres	No, far too small for a light microscope	No, they need living host cells	Antivirals. Antibiotics do nothing	One to fourteen days	Influenza, measles, COVID-19
Fungi	Cells with a nucleus and a chitin cell wall	2 to 10 micrometres (yeasts), longer threads (moulds)	Poorly. A KOH prep or a fungal stain is used	Yes, on fungal media such as Sabouraud agar	Antifungals	Days to weeks	Thrush, athlete's foot, histoplasmosis
Protozoa	One cell, with a nucleus (eukaryote)	10 to 50 micrometres	Not by Gram. Seen on a stained smear or wet mount	Special media only, often not cultured at all	Antiprotozoal drugs	Days to weeks	Malaria, giardiasis, amoebic dysentery
Parasitic worms	Many cells, a whole animal (helminth)	Millimetres to metres	No. Eggs are found in stool under a microscope	Not cultured	Antiparasitic (anthelmintic) drugs	Weeks to months	Tapeworm, hookworm, roundworm

Table 2: treatment and prognosis for the realistic candidates

Organism	Class	First-line treatment	Prognosis and what it depends on	How it usually reaches people
Listeria monocytogenes	Bacterium	Ampicillin, often with gentamicin for severe or invasive disease	Dangerous in pregnancy, in newborns, over 65, and with a weakened immune system. Invasive disease is serious and treated in hospital.	Food-borne, including deli meats, soft cheeses and unwashed produce

Staphylococcus aureus	Bacterium	Depends entirely on the susceptibility test. Methicillin-sensitive strains take a beta-lactam such as cefazolin; MRSA does not, and is treated with drugs such as vancomycin	Ranges from a minor skin infection to life-threatening bloodstream infection. The susceptibility result changes the drug, so the lab result changes the prognosis.	Skin and nose carriage, wounds, healthcare contact
Streptococcus pyogenes	Bacterium	Penicillin or amoxicillin. Still reliably sensitive	Good with treatment. Untreated infection can lead to rheumatic fever, which is why a sore throat gets cultured.	Person to person by droplets
Pseudomonas aeruginosa	Bacterium	An antipseudomonal drug such as piperacillin-tazobactam or ceftazidime. Many ordinary antibiotics do not work	Often affects people already in hospital or with weakened defences. Resistance is common, so treatment follows the susceptibility test.	Water, moist surfaces and equipment
Salmonella enterica	Bacterium	Usually fluids and supportive care. Antibiotics are reserved for severe or invasive illness	Most healthy people recover. Antibiotics are not automatic, because they are not always helpful.	Food-borne, including poultry, eggs and produce
Escherichia coli, Shiga toxin-producing	Bacterium	Fluids and supportive care. Antibiotics are generally avoided, because they may raise the risk of a serious kidney complication	A case where the reflex answer is the wrong answer. This is why naming the organism matters before choosing a drug.	Food-borne, including undercooked beef and produce
Bacillus subtilis	Bacterium	Rarely causes disease in healthy people. Usually treated as an environmental or laboratory contaminant	A hit on this organism is more often a contamination question than a diagnosis. Check your controls before you check the drug chart.	Soil, dust and laboratory surfaces

Claim ceiling. This table is a classroom reference for naming and reasoning. It is not a prescribing guide, it is not medical advice, and no one in this room diagnoses or treats anybody.

Links you need today

- **NCBI Nucleotide BLAST (blastn):** blast.ncbi.nlm.nih.gov, choose Nucleotide BLAST.
- **NCBI Taxonomy Browser:** look your organism up by name to see its classification.
- **CDC health topics A to Z:** cdc.gov, search the organism for the disease it causes.
- **MedlinePlus:** medlineplus.gov, for plain-language descriptions of an illness.

Where these tables come from

Table 1 is standard microbiology: cell structure, size ranges, staining behaviour, culture requirements and drug classes as described by the CDC and the National Library of Medicine. Table 2 follows CDC treatment guidance and standard clinical references for each named organism. Both are printed here as reference, not derived by students, which is why they sit in the index and not in a work section.

The isolate read in SEC 06 is a real 16S rRNA sequence. The identification it produces is produced by you, in BLAST, on the day. It is recorded nowhere in this packet.