

2027-04-08 · 80 minutes

Environmental data lab

Your goal: Culture a coded mock water sample on LB agar with sterile technique, so that next class you can say whether it holds bacteria and how many.

One product: Culturing record: your coded sample letter, the dilution scheme (1/10, 1/100 and 1/1,000), the volume plated (0.1 mL), the label on every plate, a completed sterile-technique checklist, your prediction of which dilution will give 30 to 300 colonies with the arithmetic behind it, and both controls named. Results stay pending until the plates are read on April 9.

Question: Can a culture detect bacterial contamination in a water sample, and how many bacteria are there?

Start the work

Use the exposure pattern to guide protection, and separate a fair funding proposal from proof of who caused the pollution.

1. Read H1, then put on goggles, gloves and a lab coat. Disinfect your bench with 10 percent bleach before any tube opens.
2. Record your coded sample letter, the tenfold scheme (1/10, 1/100 and 1/1,000), the 0.1 mL volume you will plate and control N.
3. Use Q1 and the D1 planning range to predict which dilution will give 30 to 300 colonies, before you pipette anything.
4. Build the series: 900 μ L of sterile water in three labeled tubes, 100 μ L carried forward at each step with a fresh tip, mixed each time.
5. Label four LB plates on the bottom, then spread 100 μ L of each dilution and of control N with a sterile spreader or sterile beads. No flames.
6. Tape each lid in two places, turn the plates upside down and give them to your teacher to incubate at 37°C overnight. The plates are read next class, April 9, through the closed lid.
7. Decontaminate everything that touched bacteria in 10 percent household bleach (final concentration) for at least 20 minutes before disposal, disinfect the bench again, remove gloves and wash your hands.

Save the same record locally if the route is missing. If culture supplies were missing, label the record a written plan and use the April 9 practice plate data. Open the linked BI assignment in Turn in your work.

Words you will use

culture

Bacteria grown on purpose in or on a nutrient medium under controlled conditions.

colony

A visible pile of bacteria that grew on agar from one cell or a small clump.

colony-forming unit (CFU)

A cell or clump that grows into one colony; counts are reported as CFU because one colony can start from more than one cell.

CFU per mL

Colony-forming units in each milliliter of the original sample.

LB agar

A general nutrient gel that many kinds of bacteria can grow on; it does not identify them.

E. coli K-12

A laboratory line of Escherichia coli used in teaching and research; strain HB101 is one K-12 strain.

BSL-1

Biosafety level 1, the basic practices used for well-characterized agents not known to consistently cause disease in healthy adults.

sterile

Free of living microbes.

sterile technique

Working so that only the intended bacteria reach the medium: brief lid openings, fresh tips and sterile tools.

serial dilution

A chain of dilutions by the same factor, each made from the tube before it.

dilution

The fraction of the original sample in a tube; the 1/100 tube is 0.01 of the original.

μL

Microliter, one millionth of a liter; 100 μL equals 0.1 mL.

spread plate

A plate where a small measured volume is spread evenly over the agar surface.

volume plated

The liquid volume spread on one plate, here 0.1 mL.

incubation

Holding plates at a set temperature for a set time so bacteria can grow.

negative control

A plate that should grow nothing; growth on it means contamination.

sterility check

An unopened plate incubated to show the agar itself was free of microbes.

countable range

Plates with 30 to 300 colonies, the range microbiologists typically count.

too numerous to count

A plate so crowded that colonies touch and cannot be counted reliably.

coded sample

A sample labeled with a letter so its level is unknown to the tester.

decontaminate

Kill microbes on used items, here with 10 percent bleach for at least 20 minutes.

biohazard container

A labeled container for items that touched living microbes.

prediction

A reasoned statement of what you expect before you have the result.

H1: today's question and the safety boundary

Today's question: can a culture detect bacterial contamination in a water sample, and how many bacteria are there? You grow bacteria from a coded mock water sample on LB agar today and read the plates next class, April 9.

The only organism today is *E. coli* K-12, strain HB101, a laboratory strain handled with biosafety level 1 (BSL-1) practices. Your teacher added it to sterile or boiled-and-cooled tap water to make coded samples A, B and C, plus a sterile negative control. Never culture pond water, sewage, sink or fountain water, or any sample from your own body.

Students who are immunocompromised, or sensitive to bleach or latex, tell the teacher before the lab. Wear goggles, gloves and a lab coat. Disinfect the bench with 10 percent bleach before you start and again when you finish. No food, drink or mouth pipetting. There are no open flames today; sterile spreaders or sterile glass beads do the spreading.

Decontaminate everything that touched bacteria in 10 percent household bleach (final concentration) for at least 20 minutes before disposal. Tubes, tips, spreaders and beads go into the biohazard container that holds the bleach. After incubation, plates stay taped shut and are never opened. Remove gloves last and wash your hands with soap and water.

E1: what a culture can and cannot show

One living bacterial cell that lands on agar and divides for many hours becomes a colony you can see. Two cells that land on the same spot also make one colony, so microbiologists report colony-forming units, or CFU, instead of cells.

LB agar is a general growth medium. Many kinds of bacteria grow on it, so a colony on LB shows that the sample held bacteria able to grow there. It does not tell you which kind they are.

Colonies need time. At 37°C overnight, *E. coli* colonies become easy to see; at room temperature growth is slower and takes about 48 hours. Nothing is visible today, so every result stays pending until the plates are read next class.

A crowded plate cannot be counted, and a nearly empty plate gives an unreliable number. Microbiologists typically count plates with 30 to 300 colonies. That is why you plate a dilution series instead of one guess.

CDC and NIH describe BSL-1 practices for well-characterized agents not known to consistently cause disease in healthy adults. Those practices cover this teacher-prepared K-12 strain only. They do not make an unknown or environmental culture safe to grow in class.

R1: the tenfold dilution series

A tenfold dilution mixes 1 part sample with 9 parts sterile water, so the new tube holds one tenth as many bacteria per mL. Three steps in a row give the 1/10, 1/100 and 1/1,000 tubes.

Label three tubes 1/10, 1/100 and 1/1,000 with your sample letter. Add 900 µL of sterile water to each. Carry 100 µL of your coded sample into the 1/10 tube and pipette up and down to mix.

With a fresh tip, carry 100 µL of the 1/10 tube into the 1/100 tube and mix. With another fresh tip, carry 100 µL of the 1/100 tube into the 1/1,000 tube and mix. A reused tip carries extra bacteria forward and makes a weaker tube look stronger than it is.

Keep caps closed except while you pipette. If a drop spills, cover it with a paper towel, soak the towel with 10 percent bleach, leave the bleach on for 20 minutes and tell your teacher.

R2: spread plating with sterile technique

Label four LB plates on the bottom, the agar side, near the edge: group, sample letter, dilution and date. Label the fourth plate N for the sterile negative control. A label on the lid can be swapped by accident.

Lift the lid only as far as you need, like a clamshell. Pipette 100 µL, which is 0.1 mL, onto the center of the agar. Spread it at once with a sterile spreader, or add sterile glass beads, close the lid and shake gently side to side.

Spread control N the same way, with 100 μL of the control water. If N grows colonies, bacteria other than the coded sample reached your plates, and the counts from that set cannot be trusted. Your teacher also incubates one unopened LB plate from the same batch as a sterility check on the agar.

Let the plates sit right side up for about 5 minutes so the liquid soaks in. Tip used beads into the bleach container. Tape each lid to its base in two places, turn the plates upside down and give them to your teacher.

Your teacher incubates the plates upside down at 37°C overnight, or at room temperature for about 48 hours if no incubator is available. Upside down keeps water drops from falling onto the colonies. The plates are read next class, April 9, through the closed lid.

Sterile-technique checklist: lid lifted only briefly; a fresh tip for every transfer; nothing but the spreader or beads touched the agar; tubes capped between uses; every plate labeled on the bottom; plates taped and upside down. Record any slip honestly.

D1: coded samples and planning inputs

Samples: coded samples A, B and C each contain *E. coli* K-12 HB101 added by the teacher. The sterile negative control is labeled N. Each group receives one coded sample and N.

Planning input: every coded sample was prepared to hold somewhere between 10,000 and 1,000,000 bacteria per mL. Your sample's exact level is not given; finding it is the job of the plates.

Supplied plating inputs: 100 μL , which is 0.1 mL, spread on every plate. Dilutions plated: 1/10, 1/100 and 1/1,000, plus control N. Countable range: 30 to 300 colonies per plate.

Q1: predict the countable plate

Expected colonies on a plate = bacteria per mL in the sample $\times$ dilution $\times$ volume plated in mL. Write the dilution as a fraction: the 1/100 tube is 0.01 of the original sample.

Work the low end and the high end of the D1 planning range for each of your three dilutions. Mark which plates could land in the 30 to 300 range at each end.

Write your prediction as a sentence with a reason: I predict the ____ plate will be countable because _____. Keep it in your record after the plates are read. A prediction is not a result.

P1: supplies, fallback and turn-in

If the plates, culture or sterile water are not available, do not substitute any other sample. Complete the same record as a written plan: sample letter, tube labels, volumes, plate labels, controls and prediction. On April 9 you practice the count with a labeled practice plate table.

Today's product is the culturing record. No colony count or CFU value belongs in it today, because nothing has grown yet. Mark the result line: pending, read April 9.

This lab is the Problem 4 water-testing bench work for your evidence portfolio. Ask your teacher which official activity pages it supports. Tomorrow's plate read connects your culture to the drinking-water rule for coliform bacteria.

Before this class, spend 25 minutes on Exposure pathway in Lab Notebooks when assigned, and keep that diagram with your Problem 4 work. Open the linked BI assignment in Turn in your work and submit the culturing record; save it locally if the route is missing.

M1: a worked count from a different practice sample

Practice sample Z is invented for this example; no plates were grown. Its 1/10 plate had too many colonies to count, its 1/100 plate had 64 colonies, its 1/1,000 plate had 5 colonies and its control N had none.

Only the 1/100 plate is in the 30 to 300 range. The 1/10 plate is too crowded, and the 1/1,000 plate has too few colonies to give a reliable number. The clean control supports that the plates were not contaminated during spreading.

CFU per mL = colonies ÷ (volume plated in mL × dilution). For sample Z: $64 \div (0.1 \times 0.01) = 64 \times 1,000 = 64,000$ CFU per mL.

Planning check before plating: if sample Z had held about 50,000 bacteria per mL, the 1/100 plate would be expected to show $50,000 \times 0.01 \times 0.1 = 50$ colonies, inside the countable range. The count of 64 is close to that plan, and the measured count is the one reported.

The count shows that sample Z held bacteria able to grow on LB agar. LB agar cannot show whether they were coliforms or E. coli. That question needs a coliform test, which is where April 9 goes next.

Try the decision

Your group does not know the exact level of bacteria in its coded sample and has four plates. How should you use them?

- Plate all three tenfold dilutions and control N, so at least one plate should land in the countable range.
- Put all four plates on the 1/1,000 dilution, because four copies of one plate give the most exact count.
- Replace control N with the undiluted sample, since a control plate is meant to grow nothing anyway.

Explain your choice using the reading. Discuss it or say it to yourself. This practice does not add another submission.

Your 80-minute flow

0–10 minutes	Read H1, put on goggles, gloves and a lab coat, disinfect the bench and record your coded sample letter.
10–20 minutes	Read E1, R1 and D1; record the dilution scheme and predict the countable plate with Q1.
20–40 minutes	Build the tenfold dilution series with a fresh tip at every transfer and label all four plates on the bottom.
40–55 minutes	Spread 100 μ L of each dilution and of control N; tape the plates, turn them upside down and hand them in to incubate.
55–70 minutes	Decontaminate tubes, tips and spreaders in the bleach biohazard container, disinfect the bench, wash your hands and complete the checklist.
70–80 minutes	Name both controls, mark the result pending for the April 9 plate read, and submit the culturing record.

Your response record

Use these labels on paper or in your document. The space below also works when this page is printed.

Check the safety boundary

Read H1. Put on goggles, gloves and a lab coat, disinfect the bench with 10 percent bleach, and write your coded sample letter and the organism's name in your record.

Only the teacher-prepared *E. coli* K-12 sample and control N are cultured. Ask before you touch anything else.

Use this space or the same labeled section in your existing document. Add paper when your table, graph or drawing needs more room.

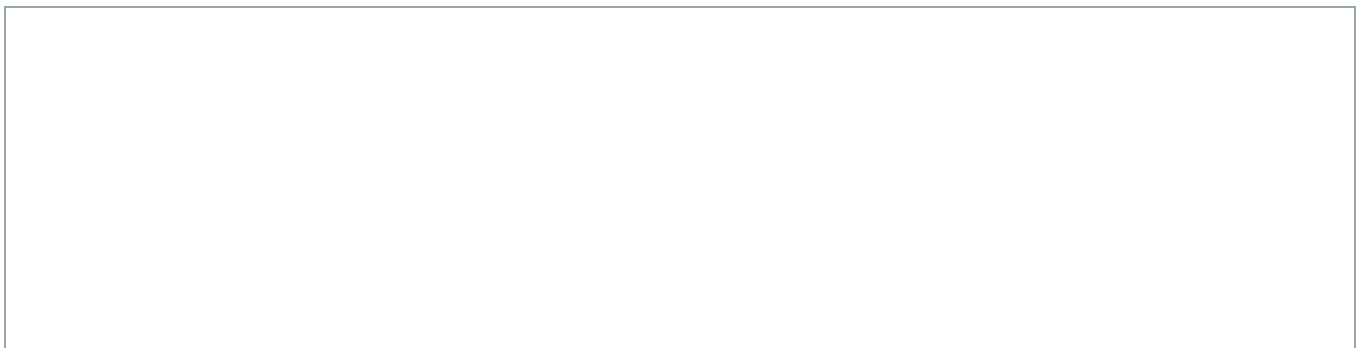


Record the dilution scheme

Use R1 and D1 to write the three tube labels, the 900 μ L and 100 μ L volumes, the 0.1 mL volume plated and all four plate labels.

Labels go on the plate bottom, not the lid.

Use this space or the same labeled section in your existing document. Add paper when your table, graph or drawing needs more room.



Predict the countable plate

Use Q1 at the low and high ends of the D1 planning range. Predict which dilution will give 30 to 300 colonies and give your reason.

Write each dilution as a fraction and keep units. Plating all three dilutions protects you if the prediction is wrong.

Use this space or the same labeled section in your existing document. Add paper when your table, graph or drawing needs more room.

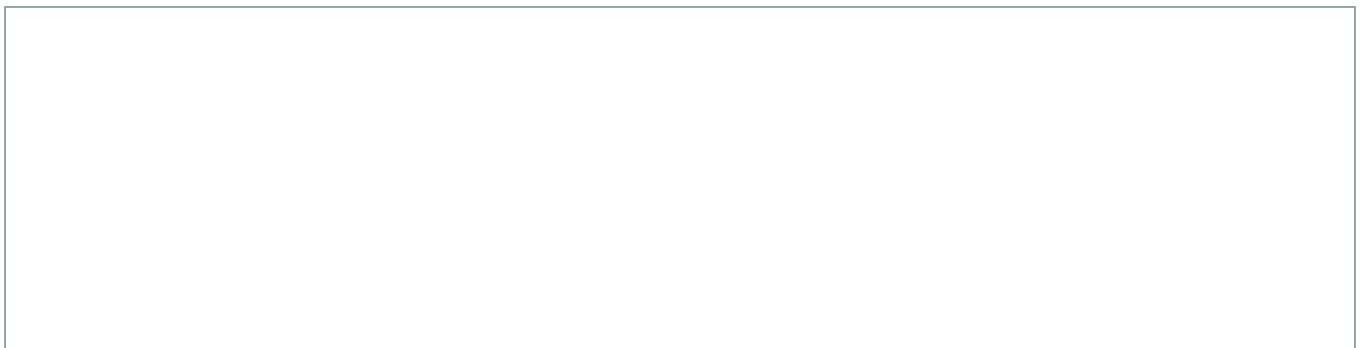


Dilute and spread the plates

Build the series with a fresh tip at every transfer, spread 100 μ L on each labeled plate, then tape the plates, turn them upside down and hand them to your teacher to incubate.

No flames. If a plate drops or opens by accident, stop and tell your teacher.

Use this space or the same labeled section in your existing document. Add paper when your table, graph or drawing needs more room.



Name the controls and check technique

Name control N and the teacher's unopened sterility-check plate, and say what growth on each would mean. Complete the R2 sterile-technique checklist honestly, including any slips.

A slip written down is useful information. A hidden slip is not.

Use this space or the same labeled section in your existing document. Add paper when your table, graph or drawing needs more room.

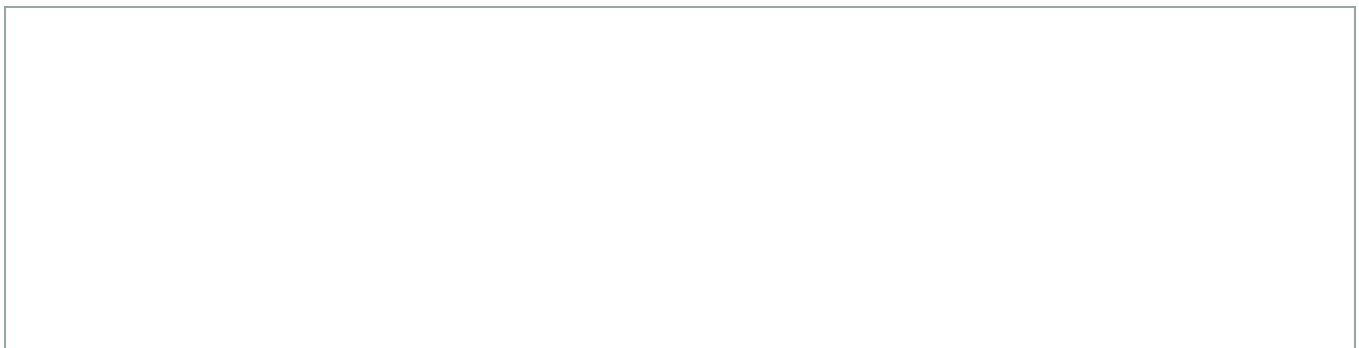


Clean up and record status

Put tubes, tips and spreaders in the bleach biohazard container for at least 20 minutes, disinfect the bench, wash your hands, and mark the result line pending until the April 9 plate read.

No colony count belongs in today's record. If supplies were missing, label the record a written plan.

Use this space or the same labeled section in your existing document. Add paper when your table, graph or drawing needs more room.



Check your product

- I recorded my sample letter, the dilution scheme, the 0.1 mL volume plated and every plate label.
- I predicted which dilution should give 30 to 300 colonies and showed the arithmetic.
- I named both controls and completed the sterile-technique checklist honestly.
- I followed the bleach cleanup and marked the result pending until the April 9 plate read.

Keep the limit: A classroom culture of a teacher-prepared *E. coli* K-12 sample shows whether bacteria grew on LB agar under these conditions. It cannot test real drinking water, identify coliforms or *E. coli*, or judge any water safe or unsafe.

Turn in your work

Submit the culturing record to the linked April 8 assignment: sample letter, dilution scheme, volume plated, plate labels, sterile-technique checklist, prediction and both controls. Mark the result pending until the April 9 plate read.

Save the same record locally if the route is missing. If culture supplies were missing, label the record a written plan and use the April 9 practice plate data. Open the linked BI assignment in Turn in your work.

[Open this assignment in Schoology.](#)

Carry forward

A culture counts only the living bacteria that grow on that medium in that time, so a count needs a countable plate, a clean negative control and a night of incubation.

Tomorrow you read your incubated plates through the closed lid, turn a colony count into CFU per mL, and ask what a drinking-water test needs that LB agar cannot give. Which of your plates do you expect to be countable?

Sources and resources

[Open today's page](#)

OPENSTAX-MICRO · Microbiology, 9.1 How Microbes Grow

OpenStax, Rice University

<https://openstax.org/books/microbiology/pages/9-1-how-microbes-grow>

BMBL · Biosafety in Microbiological and Biomedical Laboratories, sixth edition overview

CDC and NIH

<https://www.cdc.gov/labs/bmbl/index.html>