Activity 3.1.6
Gram Staining

DATE	PORTAL DAY	LESSON	TURN IN
Thursday 2026-11-30	**Source and agent CER**	**Lesson 3.1 Nosocomial Nightmare**	**CER**

> **Where this sits.** Single day, and the last day of the Lesson 3.1 case. Plates are opened today, for the first and only time.

01 Why today matters

A stain invented in 1884 sorts bacteria into two groups by a physical difference in their cell walls, and that one result changes which antibiotic a doctor reaches for.

Today you finish the case: Gram result plus cell shape plus how the cells are arranged is enough to name an organism, and then you write to the hospital.

02 What you already know

- You have your plates and a written record of colony colour, form, elevation, and margin for each patient.
- You know colony appearance narrows a field but cannot confirm an identity.
- From Unit 2 you know how to use a compound microscope and how to change objectives without crashing them into the slide.

WHAT THIS SHEET WILL NOT TELL YOU

- This sheet does not print the table that matches Gram result and cell shape to a species name. You get that in class after you have looked down the microscope and written what you saw.
- It does not tell you which organism you are looking for, what colour it will be, or what shape.
- Deciding what you expect to see and then staining is not an experiment. Stain, look, record, then match.

03 Vocabulary

TERM	WHAT IT MEANS, PLAINLY
Gram stain	A staining procedure that sorts bacteria into two groups by how much peptidoglycan is in their cell wall. Named for Hans Christian Gram, who developed it in 1884.
peptidoglycan	The mesh of sugars and amino acids that makes up a bacterial cell wall and gives it strength.
Gram positive	Bacteria with a thick peptidoglycan wall. They hold the first purple dye and finish purple.
Gram negative	Bacteria with a thin peptidoglycan wall plus an outer membrane. They lose the purple dye and pick up the pink counterstain, so they finish pink.
crystal violet	The primary stain. It is purple and it enters every cell on the slide.
Gram's iodine	The mordant. It locks onto the crystal violet and builds a complex too big to slip back out through a thick wall.

mordant	A chemical whose job is to fix a dye in place rather than to add colour itself.
decolorizer	The 95 percent ethanol step. It strips the dye complex out of thin-walled cells and leaves it trapped in thick-walled ones. This is the step that actually does the sorting.
safranin	The counterstain. It is pink, it colours the cells that lost the purple, and it is too pale to change the ones that kept it.
heat fixing	Passing a dried smear briefly through a flame so the cells stick to the glass and do not rinse away during staining.
smear	The thin film of bacteria mixed with a drop of water and spread out on a slide.
coccus	A spherical bacterial cell. Plural: cocci.
bacillus	A rod-shaped bacterial cell. Plural: bacilli. Careful, this is a shape word and it is also part of some genus names.
spirillum	A spiral bacterial cell. Plural: spirilla.
arrangement	How cells sit relative to each other after they divide. Single, in pairs, in fours, in clusters, or in chains.
diplococci	Cocci in pairs.
tetrad	Cocci in groups of four.
oil immersion	Using a drop of special oil between the slide and the 100x lens so light bends into the lens instead of scattering. Total magnification 1000x. Gram results can only be read at this magnification.
lipopolysaccharide	Written LPS. A molecule in the outer membrane of Gram negative bacteria. The body reacts hard to it, which is one reason Gram negative infections can turn dangerous fast.
septic shock	A life-threatening drop in blood pressure caused by the body's overwhelming response to an infection.

Every term on this list is flagged for the illustrated glossary, scoped to this activity only. Terms are not shared forward or backward between activities, because a definition from a later activity can hand you an answer you were supposed to derive.

04 How to do the work

1. Wash hands. Goggles, gloves, coat. Disinfect the bench. Watch your teacher demonstrate the whole procedure before you touch a reagent.
2. First, prepared slides. Get a microscope, immersion oil, and lens wipes. View at least one Gram positive and one Gram negative prepared slide, choosing slides with different cell shapes.
3. Record what you see in a table: cell shape, arrangement, Gram result, and a coloured drawing with the magnification written next to it. Do this before you make your own slides, so you know what a good result looks like.
4. Now your own smears. Write your group ID and the numbers 1, 2, 3 in PENCIL on the frosted end of three slides. Pencil survives the stains; marker does not.
5. Read this whole flame block before you light anything. Everything that needs the flame happens first, in one go, with the ethanol still in the cupboard.
6. Light the alcohol lamp. Using a clip or clothespin, pass slide 1 horizontally through the flame twice, about one second per pass, touching only the middle of the slide.
7. While the slide is still warm, add one small drop of water, roughly 20 to 50 microlitres, to the centre.
8. Open the plate. Using a sterile loop, scrape a single colony of the type that appears on all three of your plates. One colony. Not a scoop.

9. Mix that colony into the water drop with the same loop and spread it into a thin film. It should look faintly cloudy. If you can see lumps, it is too thick and it will read purple no matter what it is.
10. With a NEW loop, repeat for the second colony type on the plate, in a separate circle on the same slide.
11. Let the slide sit at room temperature for about a minute, until the water has completely dried. A wet smear will not fix and will wash off.
12. Pass the slide through the flame twice more, cells facing UP so they never touch the flame directly. This is heat fixing.
13. Repeat that whole sequence for slides 2 and 3. All three slides get fixed now.
14. Now extinguish the alcohol lamp. Look at it. Confirm it is out. Wait, look again, and only then tell your teacher your table is done with fire. Nothing flammable comes out until every lamp in the room is out and confirmed.
15. Collect the stains. Label four transfer pipettes: CV for crystal violet, I for iodine, EtOH for ethanol, S for safranin. Mixing these up ruins the slide.
16. Add two drops of crystal violet to each circle. Wait 30 seconds. Rinse gently with distilled water, either running it down the tilted slide or dipping the slide. Shake off the excess.
17. Add two drops of Gram's iodine to each circle. Wait 30 seconds. Rinse the same way. Shake off the excess.
18. Decolorize. Hold the slide at about 45 degrees over a sink or waste beaker and let 95 percent ethanol run down it in a steady stream of drops onto the centre. Stop when the liquid coming off runs clear, which is somewhere between 5 and 10 seconds. This is the step that decides your result and it is the step everyone overdoes.
19. Rinse with distilled water immediately. The rinse is what stops the ethanol from continuing to work.
20. Add two drops of safranin to each circle. Wait 30 seconds. Rinse. Shake off the excess.
21. Blot dry with paper or filter paper. Do not rub, and do not blot directly on the smear. Draw water away from the side of the drop.
22. Repeat the staining sequence for the other two slides.
23. View under the microscope. Find the cells at low power first, then step up. Add the oil and the 100x lens last. Immersion oil goes on the oil lens ONLY; putting oil under a 40x lens damages it.
24. Record for each slide and each circle: Gram result, cell shape, arrangement, and a coloured drawing.
25. Compare with your teammates. Everyone in the group stains a sample. If you all did it right, you should all get the same result. If you did not, that disagreement is data and you need to explain it.
26. Ask your teacher for the Gram reference table. Match your Gram result, shape, and arrangement against it.
27. Put your colony observations from yesterday together with your Gram results from today and write your conclusion. Then research the organism you have named: what infections does it typically cause, and how does it usually spread?
28. Compare that to what actually happened to the eleven patients and to the source you named on Friday. Does the usual mode of transmission fit the source you found? If it does not, say so; that is a real finding, not a failure.
29. Revisit the initial treatment you recommended. Does the Gram result change it? Say why.
30. Write the message to the staff at Garcia-Newgard. Short, plain, and tactful: the cause, the recommended treatment, and the steps to stop further spread.
31. Slides go into the beaker of disinfectant. Disinfect the bench, gloves off inside out, wash your hands.

05 Safety

READ EVERY LINE BEFORE YOU START

- Splash goggles, gloves, and a lab coat or apron go on before anything comes out of the cupboard, and they stay on until the bench is wiped down. Tie long hair back.
- Wash your hands with soap and warm water, then wipe the whole bench with disinfectant and let it sit wet for the time your teacher gives you. A wipe that dries in two seconds did not disinfect anything.
- The fire and alcohol rule, and this one is on us rather than on the kit. This procedure puts an open alcohol lamp and a flask of 95 percent ethanol in the same lab period, and the printed protocol does not warn you about it. So: do ALL your flame fixing first, for every slide, then extinguish the lamp, then confirm it is out, and only then does the ethanol come out of the cupboard. The ethanol bottle is never open in a room with a lit flame.
- 95 percent ethanol burns with a flame that is nearly invisible in a bright room. You would not see it until it reached something that made smoke. That is why the rule above is absolute rather than a suggestion.
- Long hair tied back, sleeves rolled, nothing loose over the bench, and nothing paper near the lamp. Know where the nearest fire blanket and extinguisher are before you strike the striker.
- You are handling living bacteria. Nothing in the kit is meant to make you sick, but treat every plate, loop, and slide as if it could. Nothing goes near your face. No food, no drink, no gum, no vape, no phone on the bench.
- You are opening live plates today. Open the lid only as far as the loop needs, hold the lid over the plate, take your one colony, and close it. Do not leave a plate open while you look for something.
- Crystal violet, iodine, and safranin stain skin, clothes, floors, and counters permanently. Keep every stain step over the sink or a waste beaker. If it goes on your hands you will be explaining it for a week.
- Slides that have carried live bacteria go into the disinfectant beaker, not the sharps bin and not the trash. Broken glass goes to your teacher.
- Wipe the bench with disinfectant again at the end. Peel your gloves off inside out without touching the outside, drop them in the hazardous waste container, and wash your hands last. Gloves are the dirtiest thing you touched all period.

06 Where students get stuck

- Over-decolorizing. This is the number one error in this lab, by a wide margin. Leave the ethanol on too long and a thick-walled cell loses its purple and reads pink, so a Gram positive organism is reported as Gram negative. Watch the runoff, not the clock; stop when it runs clear.
- Under-decolorizing. Too short and everything on the slide stays purple, including the cells that should have gone pink. If every single thing on your slide is purple, suspect your ethanol step before you believe your result.
- A smear that is too thick. Scooping half a colony gives you a pile of cells that traps dye and reads purple everywhere. One colony, thin film, faintly cloudy.
- Staining a wet smear. If the water has not fully dried before the second flame pass, the cells never fix and they rinse straight off. You will spend twenty minutes staining an empty slide.
- Putting oil on the 40x lens. That lens is not sealed for it. It damages the objective and it is expensive.
- Reading a Gram result at 400x. You cannot. Gram results are read at 1000x under oil, and any call you make below that is a guess.
- Assuming a disagreement inside your group means somebody is bad at this. It might mean two people sampled two different colonies, or one person over-decolorized. Work out which before you decide whose result to trust.

- Treating the reference table as the finish line. It gets you to a name that fits. A hospital would still run more tests, and you should say so in your message.

07 What you turn in

CER

CER, one per team. Claim: the identity of the organism causing the outbreak. Evidence: your colony observations from Wednesday and your Gram result, cell shape, and arrangement from today, stated as observations, plus how they narrow against the reference table. Reasoning: why that combination points to that organism and not to the others it could have been. Attach your message to the Garcia-Newgard staff, which must name the cause, the recommended treatment, and the steps to stop the spread, in under 200 words. Your stain tables and drawings go in your notebook.

08 Check yourself

- [ ] Which single step in the Gram stain actually separates the two groups, and what is physically happening to the cell wall during it? (Vocabulary and step 18)
- [ ] Every cell on your slide came out purple. Name two different mistakes that produce that result. (Where students get stuck)
- [ ] Why does the alcohol lamp have to be out and confirmed out before the ethanol comes out of the cupboard? (Safety)