

The Gram Stain

What it does, how it does it, and what it cannot tell you. Four steps, one deciding moment, and a 140 year old test that still changes what a doctor prescribes tonight.

USE IT IN MI Thursday SEC 04	AND IN PBS Activity 3.1.6	STEPS 4
TIME TO RUN about 5 minutes	AGE OF THE METHOD since 1884	READ WITH your circuit packet

YOUR CHARGE TODAY

By the end of this you will be able to explain, without the page, why one cell keeps the purple dye and the other loses it, and why rushing one step turns a correct answer into a wrong one.

YOU TURN IN

- Nothing. This is a reference. Keep it beside you at the microscope.

Time: Read before SEC 04. Keep it open during.

WHY THIS MATTERS

A patient arrives with a serious infection and the culture will not name the organism for another day or two. The Gram stain takes about five minutes and costs pennies, and it splits the bacterial world roughly in half. That split is often enough to choose the first antibiotic, and in a serious infection the first antibiotic is the one that decides the outcome.

READ THIS

Hans Christian Gram published this in 1884 and it is still run, unchanged in principle, in every hospital laboratory in the world. Almost nothing else in medicine has survived that long.

The question it answers is narrow and useful: what kind of wall did this bacterium build around itself? That sounds like a detail. It is not. The wall decides which antibiotics can reach the cell at all, which is why the answer changes the prescription.

The question it does not answer: which species this is. The stain gets you to a family. Only a culture, a biochemical panel, or DNA gets you to a name.

WHY THIS MATTERS

Antibiotics like penicillin work by sabotaging the building of the cell wall. That is why the wall is not a detail: the wall is the target, and the shape of the target decides which weapon reaches it.

READ THIS

Every bacterium wraps itself in a mesh called **peptidoglycan**. It is a single enormous molecule, a net of sugar chains stitched together by short protein links, and it is what stops the cell bursting under its own internal pressure.

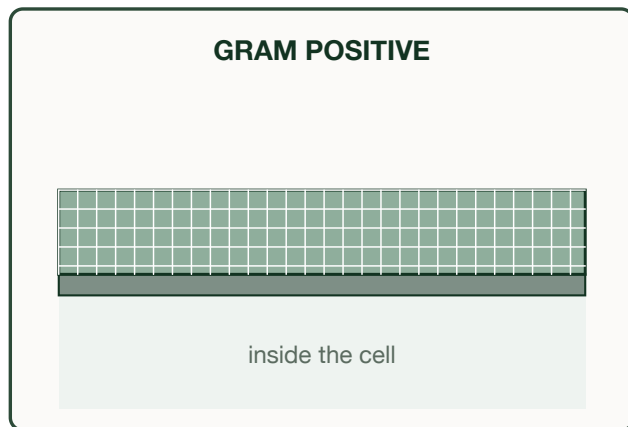
Bacteria build that mesh in one of two arrangements, and that is the entire basis of the test.

- **Gram positive:** one very thick mesh, many layers deep, sitting directly on the cell membrane. Nothing on top of it.
- **Gram negative:** a thin mesh, only a layer or two, with a second greasy membrane built on top of it. That extra layer is called the **outer membrane**, and it is made largely of fat.

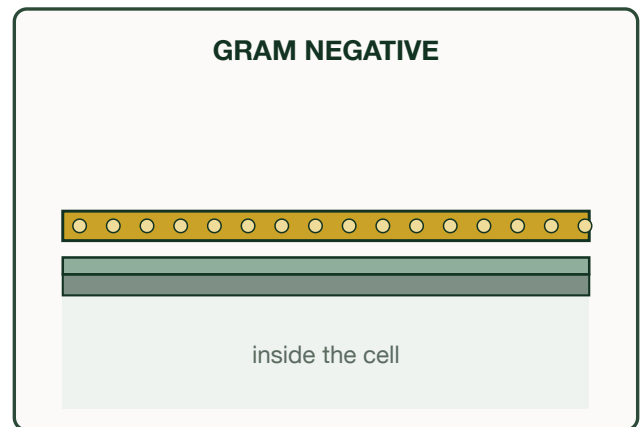
Hold on to the word **greasy** for the outer membrane. It is the reason step 3 works.

THE WHOLE TEST RESTS ON THIS ONE DIFFERENCE

Bacteria build their walls in two ways. Everything the Gram stain does follows from which way a cell built its wall.



ONE thick mesh (peptidoglycan), many layers deep. No outer membrane. Think of a thick felt blanket.



A THIN mesh, plus an extra OUTER MEMBRANE on top. Think of a thin net wrapped in a greasy plastic bag.

The two envelopes in cross section. It does not show scale, individual molecules, or the many bacteria that fit neither pattern cleanly.

WHY THIS MATTERS

Step 3 takes between five and ten seconds and it is the step that decides your result. More laboratory Gram stains are misread because of that one step than for every other reason combined.

READ THIS

Four reagents, in this order, with a gentle water rinse between each. Read across the table: for the first two steps the two cells behave identically, which is exactly why the third step matters so much.

THE FOUR STEPS, AND WHAT EACH ONE DOES INSIDE THE CELL

Read across. The third step is the only one that treats the two cells differently, and it is the step everybody rushes.

1

CRYSTAL VIOLET

30 seconds, the dye



positive



negative

A purple dye floods in. BOTH cells take it up. At this point you cannot tell them apart.

2

GRAM'S IODINE

30 seconds, the mordant, a locking agent



positive



negative

Iodine grips the purple dye and the two lock together into one bigger molecule, too big to slip out easily. Still both purple.

3

95% ETHANOL

5 to 10 seconds, the decolorizer



positive



colourless

THE DECIDING STEP. Alcohol dries the thick mesh so its holes squeeze shut, trapping the dye. On the other cell it dissolves the greasy outer membrane, and the thin mesh under it cannot hold the dye, which washes straight out.

4

SAFRANIN

30 seconds, the counterstain



positive



negative

A pink dye floods in. The colourless cell finally takes a colour and turns pink. The purple cell is already full of dye, so the pink does not show on it.

What each step does inside each cell. It does not supply the result of your own slide, and the colours drawn are the expected ones, not a guarantee.

WHY THIS MATTERS

This is the paragraph worth memorising, because from it you can rebuild every other fact about the Gram stain, including all the ways it goes wrong.

CONTROL GROUP · ALREADY SET UP FOR YOU

Why the thick-walled cell keeps the purple. Alcohol pulls water out of the thick mesh. As the mesh dries it shrinks, its holes close, and the dye-and-iodine bundle already sitting inside is now too big to get out. The alcohol traps the dye by tightening the net around it.

Why the thin-walled cell loses it. That cell's outer membrane is largely fat, and alcohol dissolves fat. The outer membrane comes apart, and underneath it there is only a thin mesh with wide holes and nothing to hold on to. The dye bundle washes straight through and the cell is left colourless, ready to take the pink counterstain.

In one sentence: the same alcohol tightens one wall shut and dissolves the other one open.

READ THIS

Now the most common error, which follows directly from the paragraph above.

Leave the alcohol on too long and it keeps pulling water out of even the thick mesh, until that wall gives up its dye too. A genuinely Gram positive organism then reads as Gram negative. This is called **over-decolorizing**, and it is why the sheet tells you to stop when the liquid running off the slide turns clear rather than counting to a number.

The opposite error exists too. Too little alcohol leaves the thin-walled cell still holding dye, and a Gram negative organism reads as Gram positive.

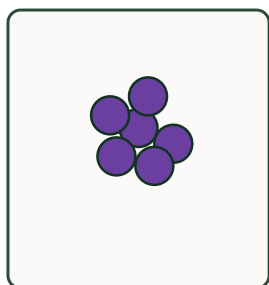
One more: an old culture works badly. As Gram positive cells age their walls break down, and an old colony can read negative when a fresh one from the same plate reads positive. Always stain from a fresh growth.

WHY THIS MATTERS

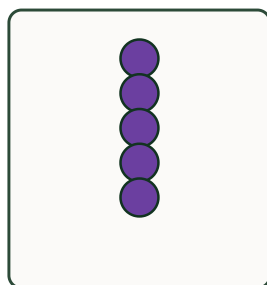
A report that says gram-positive cocci in clusters already points a physician at a short list of organisms, hours before anything has grown. That is the whole clinical value of five minutes at a microscope.

THE STAIN SHOWS YOU TWO MORE THINGS AT THE SAME TIME

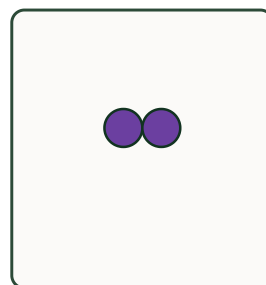
Colour is only half the answer. The same slide tells you the shape of the cells and how they hang together, and those two narrow the field as hard as the colour does.

**CLUSTERS**

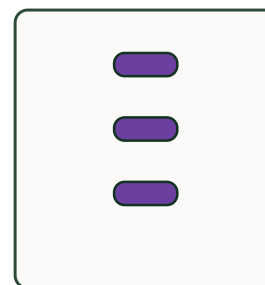
staphylo-

**CHAINS**

strepto-

**PAIRS**

diplo-

**RODS**

bacilli

A round cell is a coccus, plural cocci. A rod is a bacillus, plural bacilli. So purple round cells in clusters is reported as gram-positive cocci in clusters, which is already a short list of organisms.

Shape and arrangement. It does not show every shape bacteria take, and the arrangement you see depends partly on how the smear was spread.

READ THIS

Report all three things together, in this order: the Gram reaction, the shape, then the arrangement.

"Gram positive cocci in chains" is a real finding. "Purple" is not.

HOW THE THREE FINDINGS COMBINE

What you see	How you report it	What it narrows to
Purple, round, in grape-like clumps	('gram-positive cocci in clusters',)	('a short list including the staphylococci',)
Purple, round, in chains	('gram-positive cocci in chains',)	('a short list including the streptococci',)
Purple, rod shaped	('gram-positive bacilli',)	('a different short list again',)
Pink, rod shaped	('gram-negative bacilli',)	('a large group including many gut organisms',)
Pink, round, in pairs	('gram-negative diplococci',)	('a short and clinically urgent list',)

WHY THIS MATTERS

Knowing what a test cannot do is what separates someone who runs a test from someone who can be trusted with the result.

CONTROL GROUP · ALREADY SET UP FOR YOU

What a Gram stain can establish

- That bacteria are present in the sample, and roughly how many.
- Which broad wall type they have, which narrows the antibiotic choice today.
- Their shape and how they group, which narrows the field further.
- Whether there are white blood cells present, which suggests real infection rather than contamination.

What it cannot establish

- The species. Purple cocci in clusters is a family, not a name.
- Whether the organism is causing the illness. Bacteria live on us everywhere.
- Which antibiotic will actually work. That needs a susceptibility test, and for some organisms the susceptibility result changes the drug completely.
- Anything at all about viruses, which have no cell wall and do not appear.

READ THIS

Your controls, and why they are not optional. A stain control slide carries known Gram positive and known Gram negative organisms side by side. If both colours do not appear on the control, your reagents or your timing failed and your patient slide means nothing, no matter how convincing it looks. Check the control first, every time, before you look at anything else.

CHECK YOURSELF · YOU COMPLETE THIS

In your own words, why does alcohol trap the dye in one cell and release it from the other?

A slide reads gram-negative. Name two different ways that result could be wrong.

Your report says gram-positive cocci in clusters. Write one sentence on what a doctor can do with that, and one on what they still cannot do.

The words in this document

- **Peptidoglycan:** the mesh that forms a bacterial cell wall. Sugar chains stitched together by short protein links.
- **Outer membrane:** the extra fatty layer that sits on top of the thin mesh in Gram negative bacteria.
- **Mordant:** a chemical whose job is to fix a dye in place rather than to add colour. Gram's iodine is the mordant here.
- **Decolorizer:** the alcohol step that removes dye from cells that cannot hold it.
- **Counterstain:** a second dye, a different colour, that shows up the cells the first dye left behind.
- **Over-decolorizing:** leaving the alcohol on too long, so a Gram positive organism reads negative.
- **Coccus** (plural cocci): a round bacterium. **Bacillus** (plural bacilli): a rod-shaped one.
- **Staphylo-:** in clusters. **Strepto-:** in chains. **Diplo-:** in pairs.

Where this comes from

Standard clinical microbiology: the structure of the Gram positive and Gram negative cell envelope, the role of the crystal violet and iodine complex, alcohol dehydration of thick peptidoglycan and dissolution of the outer membrane, and the recognised error modes of over-decolorizing and staining from aged cultures. Described in the same terms by the CDC and the National Library of Medicine, and in any clinical microbiology text.

The method dates to Hans Christian Gram, 1884. The colours and the order of reagents have not changed.

Claim ceiling

Everything here describes a laboratory method. Nothing in this document is a diagnosis or medical advice, and no result produced in this classroom describes any real person.