

Exercise walk-through

8.1

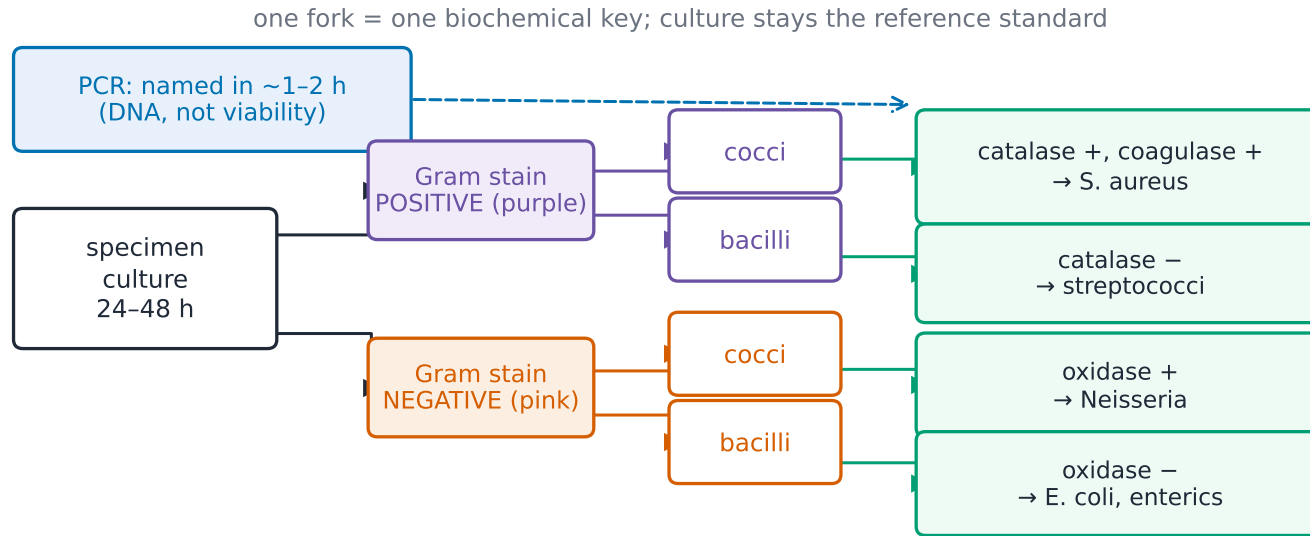
Classification and the diagnostic laboratory

University Medicine

Classification and the diagnostic laboratory: fork by fork

- 1 Gram chemistry** Crystal violet-iodine trapped by thick **peptidoglycan** → purple; thin wall + outer membrane loses it → pink counterstain.
- 2 Report walk** Gram-positive cocci in clusters; catalase + (not streptococci); coagulase + → *Staphylococcus aureus*.
- 3 Fast lane PCR** names DNA in ~1-2 h (meningitis); culture 24-48 h proves viability and susceptibility.

The classification tree from specimen to named organism



Gram stain first, then shape, then biochemistry; **PCR** is the fast lane.

Exercises 2.1-2.3

2.1 Why do Gram-positive cells keep the purple complex? [3]

2.2 Gram-positive cocci in clusters, catalase negative – which genus? [3]

2.3 Name the one thing PCR cannot tell you that culture can. [3]

Exercises 2.1-2.3

2.1 Why do Gram-positive cells keep the purple complex? [3]

2.1

- Thick peptidoglycan (many layers, teichoic acids) traps the crystal violet–iodine complex at the decolouriser step.

2.2 Gram-positive cocci in clusters, catalase negative – which genus? [3]

2.2

- Streptococci – catalase negative rules out staphylococci ✓.

2.3 Name the one thing PCR cannot tell you that culture can. [3]

2.3

- Whether the organism is ALIVE and drug-sensitive – viability and susceptibilities come only from culture ✓.

Exercise 3.1

3.1 Cerebrospinal fluid from a fictional patient with suspected meningitis: PCR positive for *S. pneumoniae* at 90 minutes; culture still negative at 12 hours.

Explain the chemistry behind the speed difference (amplified nucleic acid vs growing organisms). State what the PCR result cannot tell you (viability, susceptibility). Explain why culture must still be followed to completion. [7]

Exercise 3.1

3.1 Cerebrospinal fluid from a fictional patient with suspected meningitis: PCR positive for *S. pneumoniae* at 90 minutes; culture still negative at 12 hours.

Explain the chemistry behind the speed difference (amplified nucleic acid vs growing organisms). State what the PCR result cannot tell you (viability, susceptibility). Explain why culture must still be followed to completion. [7]

3.1

- PCR amplifies nucleic acid present in the sample, dead or alive, so a template answers in ~1–2 h.
- Culture must grow viable cells to visible colonies – 24–48 h for most bacteria.
- PCR cannot report viability or susceptibility categories ✓.
- Culture yields the isolate for susceptibility testing and the reference-standard identification.

Exercise 3.2

3.2 Two blood-culture bottles from one fictional patient: bottle A grows coagulase-negative staphylococci at 24 h; bottle B shows no growth at 48 h.

Use contaminant biology to interpret the pair (skin flora vs true bacteraemia usually hits both bottles). State what should be done before any organism is named from a single positive bottle.

Exercise 3.2

3.2 Two blood-culture bottles from one fictional patient: bottle A grows coagulase-negative staphylococci at 24 h; bottle B shows no growth at 48 h.

Use contaminant biology to interpret the pair (skin flora vs true bacteraemia usually hits both bottles). State what should be done before any organism is named from a single positive bottle. [7]

3.2

- Coagulase-negative staphylococci are skin flora – the usual contaminant of venepuncture ✓.
- True bacteraemia typically seeds both bottles; one positive of two points to contamination.
- Repeat the culture with careful skin preparation before naming any organism from a single bottle.