

SI Microbiology - Full Discipline Demo

Bacterial Morphology and Staining Techniques

Final Report - Answer Guide

Institution	Science Interactive University
Session	SI Microbiology - Full Discipline Demo
Course	SI Microbiology - Full Discipline Demo
Instructor	Sales SI Demo

Test Your Knowledge

Match each term with the best description.

Terms to match:

- ❖ Coccus
- ❖ Lipopolysaccharides
- ❖ Spirillum
- ❖ Peptidoglycan
- ❖ Streptobacilli

Descriptions to match:

- 1 A spherical bacterial cell with a diameter of approximately 0.5 μm
- 2 Rod-shaped bacterial cells linked to form a chain
- 3 A helical-shaped bacterial cell approximately 15 μm in length
- 4 Specialized molecules of the outer cell membrane that function as endotoxins
- 5 Mesh-like polymer present in bacteria cell walls

Correct answers:

- 1 Coccus 2 Streptobacilli 3 Spirillum 4 Lipopolysaccharides
5 Peptidoglycan

Categorize each statement as related to direct simple staining, negative staining, or Gram staining.

⚡ Does not require heat fixing of smears.

⚡ All cells appear dark on a light background.

⚡ Cells stain purple or pink depending on cell wall structure.

⚡ All cells appear clear on a stained background.

⚡ Typical stains include crystal violet, methylene blue, and malachite green.

⚡
Four-step, differential staining technique of applying purple and red dyes, binding agents, and releasing agents to the cell walls of bacteria.

Simple Direct Staining	Negative Staining	Gram Staining
1	2	3

Correct answers:

1 All cells appear dark on a light background.

Typical stains include crystal violet, methylene blue, and malachite green.

2 Does not require heat fixing of smears.

All cells appear clear on a stained background.

3 Cells stain purple or pink depending on cell wall structure.

Four-step, differential staining technique of applying purple and red dyes, binding agents, and releasing agents to the cell walls of bacteria.

Exploration

A ____ is an arrangement of coccus cells.

- ☐ streptobacillus
- ☒ tetrad ✓
- ☐ spirillum
- ☐ spirochaete

Negative staining results in a colorless cell on a stained background.

- ☒ True ✓
- ☐ False

Gram-positive cells have a thick outer cell wall composed of peptidoglycan.

- ☒ True ✓
- ☐ False

____ washes dyes from Gram-negative bacteria.

- ☐ Crystal violet
- ☐ Iodine
- ☒ Decolorizer ✓
- ☐ Safranin

Exercise 1

Did both the crystal violet and Congo red stains adhere to the *S. cerevisiae* cells? Reference the functions of simple direct and negative stains and your results recorded in Photos 2-3 in your explanation.

Only the crystal violet stain adhered to the *S. cerevisiae* cells and functions as a simple direct stain. In Photo 2, the crystal violet stained cells appeared a purple color on a light background.

Congo red was repelled by the cells and functions as a negative stain that dyes the background, leaving the cells unstained. In Photo 3, the cells appeared clear on a red/orange background.

Which of the staining methods in this exercise required heat fixing? What are the advantages and disadvantages of the heat fixing technique?

The simple direct staining method required heat fixing whereas the negative staining method did not. Heat fixing is required to both kill the cells and adhere them to the slide. The cells would have been rinsed off the slide with the stain if not heat fixed. The disadvantage of heat fixing is that it shrinks the cells altering their appearance and size.

Photo 1: Simple Direct and Negative Stained Slides
(SAMPLE ANSWER BELOW)



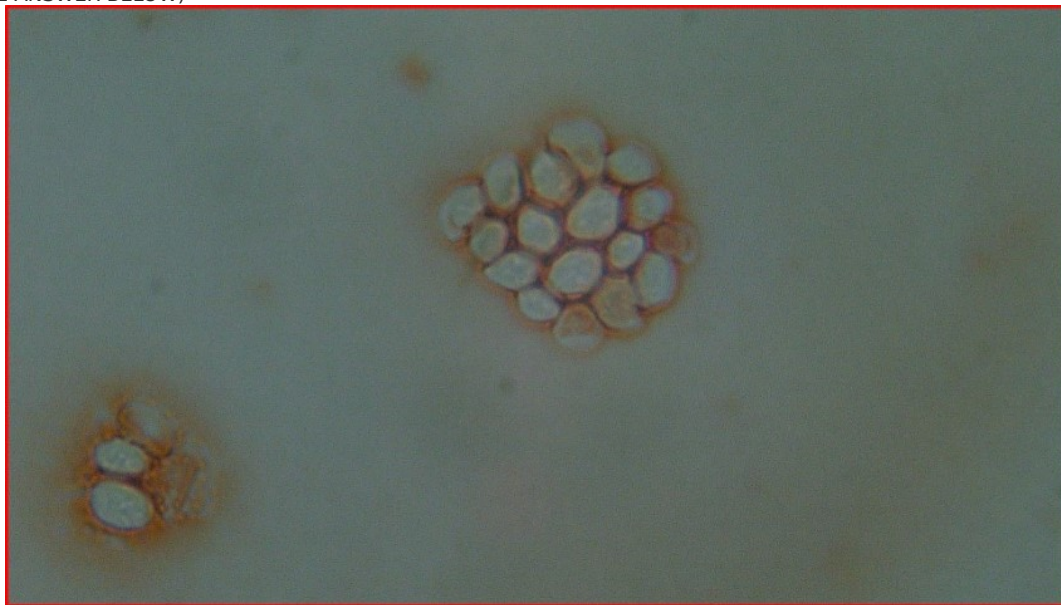
Photo 2: *S. cerevisiae* Direct
(SAMPLE ANSWER BELOW)



Data Table 1: Magnification of *Saccharomyces cerevisiae* Slides
(SAMPLE ANSWER BELOW)

Slide	Magnification
<i>Saccharomyces cerevisiae</i> Direct	1500x (600x V-scope)
<i>Saccharomyces cerevisiae</i> Negative	1500x (600x V-scope)

Photo 3: *S. cerevisiae* Negative
(SAMPLE ANSWER BELOW)



Exercise 2

How do the three bacteria that you stained in this exercise differ in cell shape and arrangement? Reference Photos 5-7 in your explanation.

S. epidermidis cells are coccus shaped and occur in small clusters termed staphylococci arrangements as recorded in Photo 7. Both *B. subtilis* and *E. coli* cells are rod shaped and occur as single cells termed bacilli as recorded in Photos 5 and 6.

How does the cell wall structure of the three bacteria species differ? Reference the results recorded in Data Table 2 and Photos 5-7 in your explanation.

The cell walls of *S. epidermidis* and *B. subtilis* contain multiple, thick layers of peptidoglycan and teichoic acids resulting in the purple staining from the retention of the crystal violet dye during the Gram staining process. These cells appear purple as recorded in Photos 7 and 5 respectively. *S. epidermidis* and *B. subtilis* are Gram + as recorded in Data Table 2. The cell walls of *E. coli* are thin, comprised of a single layer of peptidoglycan sandwiched between an outer and inner cell membrane resulting in crystal violet being washed away and staining by safranin during the Gram staining process. These cells appear pink as recorded in Photo 6. *E. coli* are Gram - as recorded in Data Table 2.

Photo 4: Gram Stained Slides
(SAMPLE ANSWER BELOW)

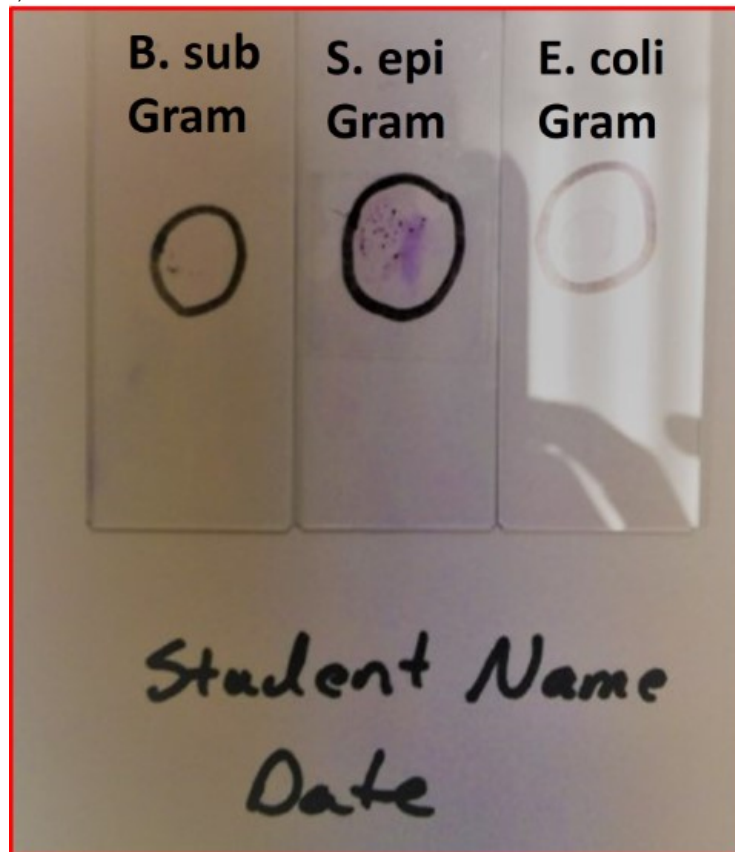
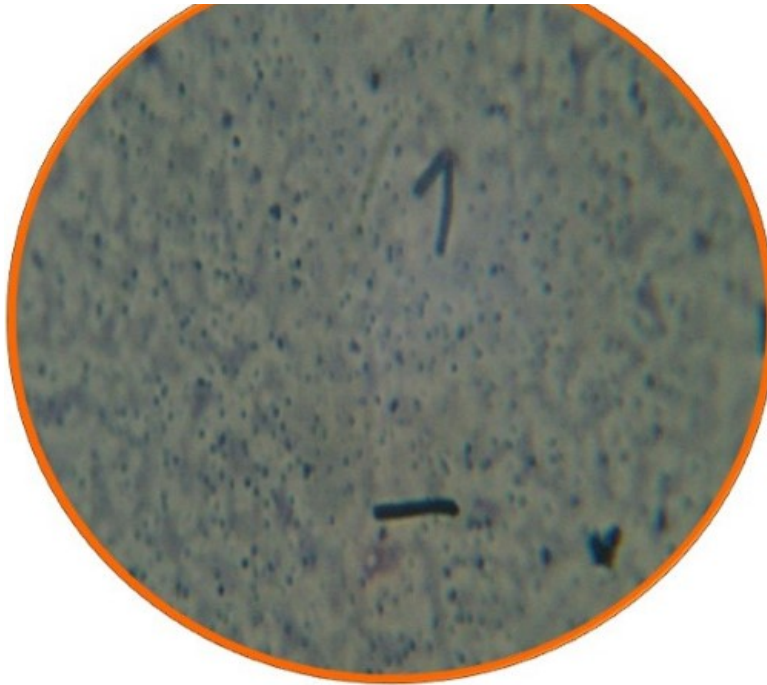


Photo 5: B. subtilis Gram Stain
(SAMPLE ANSWER BELOW)





Data Table 2: Gram Staining Results
(SAMPLE ANSWER BELOW)

Slide	Magnification	Gram +/-
<i>B. subtilis</i>	1500x (600x V-Scope)	Gram +
<i>E. coli</i>	1500x (600x V-Scope)	Gram -

S. epidermidis

1500x (600x V-Scope)

Gram +

Photo 6: E. coli Gram Stain
(SAMPLE ANSWER BELOW)

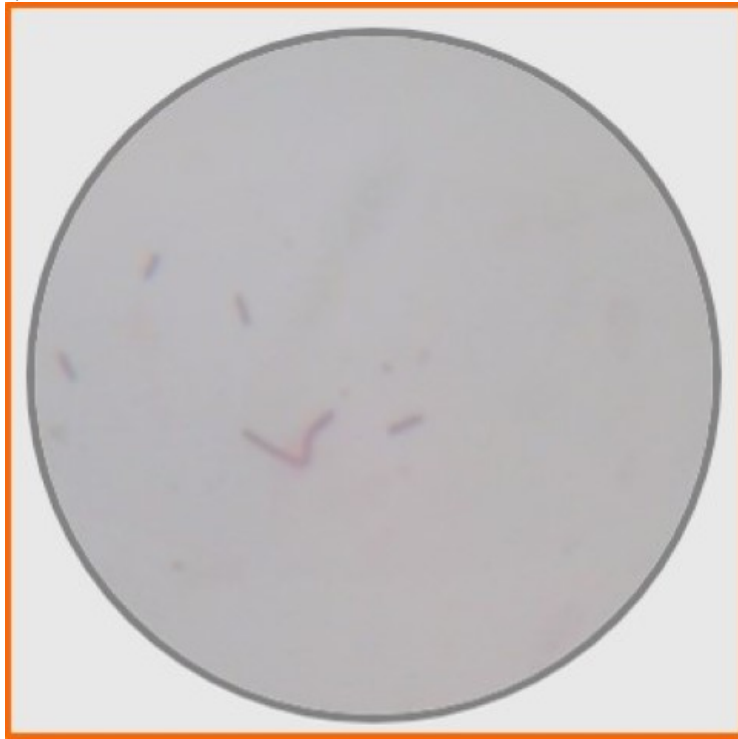
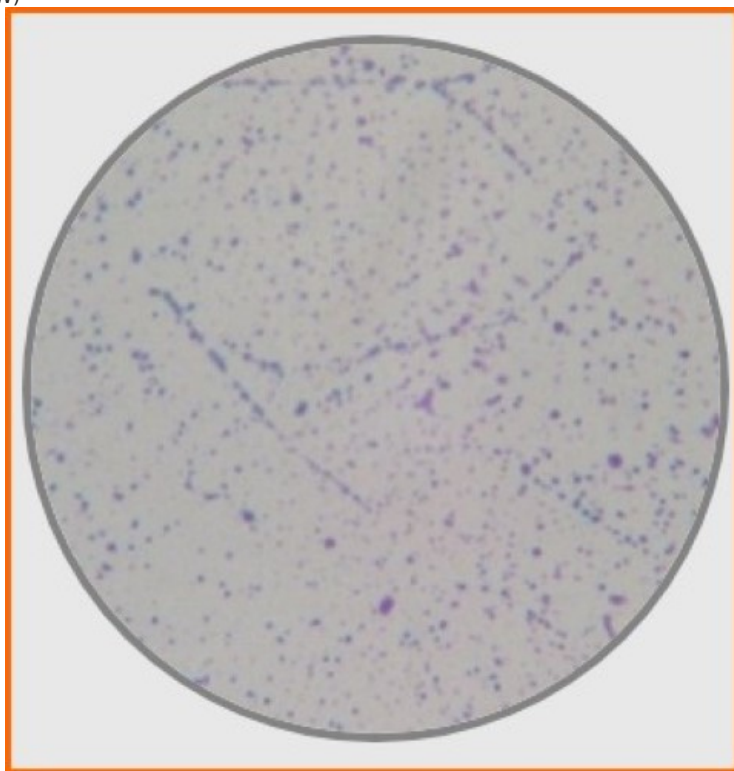


Photo 7: *S. epidermidis* Gram Stain
(SAMPLE ANSWER BELOW)



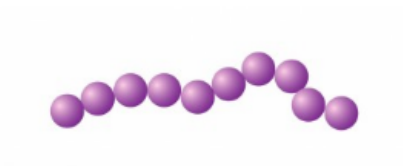
Competency Review

_____ exist only as single cells and do not form clusters or chains.

- ☐ Bacilli
- ☐ Cocci
- ☒ Spirilla
- ☐ Sarcina

✓

The cell arrangement pictured below is _____.



- ☐ staphylococcus
- ☒ streptococcus
- ☐ diplobacillus
- ☐ spirochaete

✓

Differential staining uses two or more dyes to stain structural components of a cell.

- ☒ True
- ☐ False

✓

Congo red is an example of a(n) _____ stain.

- ☒ acidic
- ☐ basic
- ☐ neutral
- ☐ differential

✓

____ stains Gram-negative bacteria pink.

- ☐ Crystal violet
- ☐ Iodine
- ☒ Safranin
- ☐ Congo red

Gram-positive bacteria have an outer cell membrane containing endotoxins.

- ☐ True
- ☒ False

Saccharomyces cerevisiae smears must be heat fixed after staining with crystal violet.

- ☐ True
- ☒ False

Slides should be rinsed with water after applying ____ during the Gram staining process.

- ☐ crystal violet
- ☐ iodine
- ☐ decolorizer
- ☒ All of the above

E. coli appear as ____ when Gram stained.

- ☐ purple cocci
- ☒ pink rods
- ☐ purple spirals
- ☐ pink tetrads

Extension Questions

A microbiology student Gram-stained the yeast, *S. cerevisiae*, producing a sample with cells colored purple, pink, brown, and green. Explain why this organism produced results that deviate from typical purple or pink Gram-stained cells. (SAMPLE ANSWER BELOW)

Yeasts are single-celled eukaryotes, unlike bacteria which are prokaryotes. The cell wall structure of yeasts, therefore, significantly differs from that of bacteria. Eukaryotic cell walls do not contain the polymer peptidoglycan which reacts with and retains the crystal violet stain used in Gram-staining, and therefore do not respond to Gram-staining like bacterial cells.