

Gram Stain Interpretation Guideline

(NB Provincial Health Authorities Anti-Infective Stewardship Committee, October 2020)

Morphology	Most Likely Organisms
Gram positive	
Cocci in clusters or clumps	<i>Staphylococcus aureus</i> Coagulase negative <i>Staphylococci</i> - Ex. <i>S. epidermidis</i> , <i>S. lugdunensis</i> , <i>S. hominis</i> , <i>S. warneri</i> , <i>S. saprophyticus</i> , <i>S. haemolyticus</i> , etc. <i>Micrococcus</i> <i>Aerococcus</i>
Cocci in pairs or chains	<i>Streptococcus pneumoniae</i> (classically Gram positive diplococci) Viridans Group <i>Streptococci</i> , - Ex. <i>S. mitis</i> , <i>S. sanguinis</i> , <i>S. salivarius</i> , <i>S. oralis</i> , <i>S. bovis</i> , Anginosus group (<i>S. anginosus</i> , <i>S. constellatus</i> , <i>S. intermedius</i>) Beta-haemolytic pyogenic <i>Streptococci</i> (group A, B, C and G) <i>Enterococcus</i> -Ex. <i>E. faecalis</i> , <i>E. faecium</i> <i>Rarer; Gemella</i> (sometimes observed in clusters), <i>Abiotrophia</i> , <i>Granulicatella</i> Anaerobic cocci
Bacilli – small, non-spore forming	<i>Corynebacterium</i> <i>Cutibacterium</i> (previously <i>Propionibacterium</i>) <i>Listeria</i> Anaerobic gram positive bacilli
Bacilli – large, spore or non-spore forming	<i>Bacillus</i> <i>Clostridium</i> <i>Lactobacillus</i>
Bacilli – branching/beaded/rods	<i>Nocardia</i> and other aerobic actinomycetes <i>Actinomyces</i> Nontuberculous <i>Mycobacterium</i>
Yeast or pseudohyphae	<i>Candida</i>
Gram negative	
Bacilli	Enterobacteriaceae - Ex. <i>Escherichia coli</i> , <i>Serratia</i> , <i>Klebsiella</i> , <i>Enterobacter</i> , <i>Citrobacter</i> , <i>Morganella</i> , <i>Proteus</i> , <i>Providencia</i> , <i>Salmonella</i> , etc. Some non-fermentative rods - Ex. <i>Pseudomonas</i> , <i>Stenotrophomonas</i> , etc. <i>Legionella</i> Anaerobic gram negative bacilli -Ex. <i>Bacteroides</i> , <i>Prevotella</i> , <i>Porphyromonas</i>
Coccobacilli/pleomorphic bacilli	<i>Haemophilus</i> <i>Acinetobacter</i> (can be mistaken for a Gram positive cocci) <i>Pasteurella</i> <i>Aggregibacter</i> (previously <i>Actinobacillus</i> , <i>Haemophilus aphrophilus</i>) <i>Eikenella corrodens</i> <i>Kingella</i> <i>Francisella tularensis</i> <i>Brucella</i> Other anaerobic gram negative coccobacilli
Fusiform bacilli	<i>Fusobacterium</i> <i>Capnocytophaga</i>
Curved bacilli or spiralled	<i>Campylobacter</i> <i>Vibrio</i>
Diplococci	<i>Neisseria</i> -Ex. <i>N. meningitidis</i> , <i>N. gonorrhoeae</i> <i>Moraxella catarrhalis</i>
Cocci	<i>Veillonella</i>

What is a Gram stain?

The Gram stain is a quick test that is used by the microbiology laboratory to differentiate different types of bacteria based on the biochemical properties of their cell walls. Gram staining requires the bacterial culture to be smeared to a glass slide, a violet stain is then added to the slide which stains Gram-positive bacteria violet. The slide is then rinsed with a decolorizing agent which washes away the violet stain from Gram-negative bacteria. Following this step the slide is counterstained with a red dye which colors the Gram-negative bacteria pink.

Gram-positive bacteria:

Have a thick cell wall which is made up of peptidoglycan (50-90% of cell wall). This thick peptidoglycan layer allows the organisms to retain the violet stain and prevents it from being washed away during the decolorization step.

Gram-negative bacteria:

Have a thinner layer of peptidoglycan (about 10% of the cell wall), and they lose the violet stain during the decolorization step but retain the counter stain (pink).

Why do we use it?

It helps us differentiate Gram-positive bacteria from Gram-negative bacteria which can help guide empiric treatment.

What else can the Gram stain tell us about the bacteria?

The microbiology lab also uses the morphology and arrangement of bacteria to further differentiate bacteria:

Spherical morphology = cocci

- Arrangement: chains or clusters:
- Gram-positive cocci in clusters usually indicate *Staphylococcus* species
- Gram-positive cocci in chains usually indicate *Streptococcus* or *Enterococcus* species
- Rod-shaped morphology = bacilli
 - Ex. *Escherichia*, *Klebsiella*, *Enterobacter*, *Pseudomonas*, etc.

Are there bacteria that do not show up on a Gram stain?

Some organisms are Gram-variable – they may stain either negative or positive, and some organisms are not susceptible to Gram staining, for example when there is no cell wall structure (Ex. *Mycoplasma*).

How do we know if culture is contaminated?

Any bacteria detected on Gram stain of a **sterile** site specimen should be considered significant, although a negative Gram stain does not exclude infection.

Nonsterile specimens also generally contain human cells such as epithelial cells and white blood cells. For example: a high-quality sputum specimen has high numbers of white cells and few epithelial cells; a low-quality specimen has high numbers of epithelial cells and low numbers of white cells. Low-quality sputum specimens may be rejected by the laboratory as unfit for culture

What can't a Gram stain tell us?

- How the patient is doing. Clinical status of patient in addition to Gram stain results should guide therapy.
- Presence of a multidrug-resistant organism. Assess patient risk factors, recent antimicrobial therapy and prior microbiology when considering the need to cover for multidrug-resistant organisms. For example, MRSA risk factors include: history of MRSA infection or colonization, household contact with a MRSA colonized individual, IV drug use, homelessness, incarcerated persons, recent travel to or residing in an MRSA endemic region or community.