

Gram Stains Made Easy – Part 2 Handout

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Good Source of images for training, review, or competency testing: American Society for Microbiology's Microbe Library. A free, non-copyrighted site with a good collection of images. They have also digitalized a very good Gram stain atlas: [Interpretation of Gram Stains and Other Common Microscopic Slide Preparations](#) by Fred Tenover and J.V. Hirshmann, original publication date: 1990.

Selecting the proper area to examine

- Scan on low power – 20-15 fields to get a feel for the slide
- Avoid extremely thick area – they are usually not decolorized enough
- Avoid very thin areas
- Look at the nuclei of the neutrophils, if present. They should be pink. If purple, the area is not decolorized enough and should not be used.

WBCs

- Appearance varies with slide preparation and fixation method
- Cells may look normal or be degenerating as they release the contents of their granules in the process of destroying bacteria
- Degenerating WBCs have no cytoplasm. You just can see the nuclei
- Look for intracellular bacteria – remember they may not stain properly due to the effects of the WBC activity on them

- On a Gram stain you can't really tell lymphocytes from monocytes, so just call them mononuclear cells

RBCs

- Often are distorted
- Stain as pink disks
- Often see water artifacts (look hollow)

Epithelial cells

Squamous

- Large cells with irregular shape
- Small nucleus, lots of cytoplasm

Epithelial Cells (con't)

Columnar

- Seen most often in BALs & bronchial washings
- Long thin cells with large nucleus near bottom of cell
- May have a tail
- Will have cilia present
- Important to report if seen

Cocci

Occur in various arrangements

- Pairs – *Streptococcus*, *Enterococcus*;
 - *Streptococcus pneumoniae* – lancet-shaped diplococci
 - May see the capsule, especially when there is a lot of pink background material
- “Kidney or Coffee Bean” or Hamburger bun pairings are usually *Neisseria* or *Moraxella catarrhalis*
- Chains – *Streptococcus*
 - In blood cultures
- Clusters – *Staphylococcus*
- Tetrads – *Micrococcus*
 - Tend to decolorize easily
 - Cells are larger than *Staphylococcus*

- When trying to determine if you have overdecolorized Staphs or Streps or *Neisseria*, look at shape of the pairings; compare with correct staining cells in same field.
- These organisms could be dead or dying.

- Remember *Staphylococcus* is usually seen in pairs, tetrads, clumps and clusters, NEVER CHAINS
- Remember *Streptococcus* is usually seen in pairs, short and long chains, NEVER CLUSTERS

Gram Positive Rods

- Singles or in pairs (remember, this is how they multiply)
- Pleomorphic – different sizes and shapes in the same field
- Diphtheroids – another term for the way we describe *Corynebacterium* species
- Pallisades – stacked side by side (‘picket fence’ arrangement)
- Curved – (*Mobiluncus* – NF of vaginal tract)

Gram stain morphology can help a lot with identification

Large, boxy (squarish ends) GPRs

- *Bacillus* or *Clostridium* - may see spores (only sporeformers we routinely see in the clinical micro lab)
- May stain somewhat gram variably, especially *Clostridium* due to oxygen exposure

‘Regular’ shaped GPR

- *Listeria*, *Lactobacillus*, *Erysipelothrix*
- May be short or long, but cells are uniform in size and shape
- In CSFs, *Listeria* is often intracellular, that is where it likes to live

Pleomorphic, club-shaped, letters, diphtheroidal GPRs

- *Corynebacterium*, *Propionibacterium*
- Will see a large morphological variation in shape – swollen cells, palisading, ‘picket fence’, letters (V’s Y’s)
- Swollen cells often stain variably

Branching, beaded GPRs

- Suggests *Nocardia*, *Streptomyces*, or *Mycobacteria*
- Beading is due to difference in cell wall from the typical Gram positive organisms
- Mycobacteria are hard to stain with Gram Stain but may be seen in sputum specimens

Gram Variable Rods

- *Gardnerella vaginalis* is the only true Gram variable rod. Its cell wall composition differs from other Gram positive organisms
- Associated with bacterial vaginosis and clue cells

Gram Negative Rods

- Singles or in pairs (remember, this is how they multiply)
- Safety Pin appearance – ends of the rods stain darker than the middle. In some instances it is hard to see the mid section of the rod and you may think they are Gram negative cocci
- Pleomorphic – different sizes and shapes in the same field
- Coccobacilli – rods that tend to be very small are round-up (*Haemophilus*)
- Fusiform – spindle-shaped (thicker in middle, tapers to points on ends)
- Curved or spiral (*Campylobacter*, *Vibrio*)

- *Haemophilus influenzae* – coccobacilli, very faint staining, blend in with the background, especially in sputum
- Spindle shaped rods include the genera *Fusobacterium* (an anaerobe) and *Capnocytophaga* (associated with bite wounds)
- *Cardiobacterium hominis* – exclusively associated with endocarditis so will be seen in blood culture bottles. Form rosettes on Gram stain. Very distinctive!
- *Campylobacter* – curved, rods, comma shaped, spirals/corkscrews

Fungus

Yeast and moulds

- Yeasts are most often seen in vaginal specimens and sputum
- Very large, may see hyphae and pseudohyphae as well as individual and budding cells.
- These will be seen in blood cultures, filamentous fungi rarely cause septicemias
- Filamentous fungi not usually seen in sputum because they invade the tissue. In rare cases will appear in expectorated specimens, most likely to be seen in invasive collection techniques.
- *Cryptococcus neoformans* – common cause of meningitis in immunocompromised patients. Posses a large capsule, may be seen on GS

Artifacts

- Stain precipitate – Can appear as needles (resemble GPRs) or granules (resemble GPC)
- They will be irregular in size and shape compared to actual bacteria
- Often are refractile
- Usually seen in thick areas

Vaginal Smears

- Primarily looking for yeast and clue cells
- Clue cells are squamous epithelial cells that are covered with Gram variable rods
- May see *Trichomonas*, but on a Gram stain they resemble WBCs, best to use wet mount

Male Urethral Smears

- Primarily looking for *Neisseria gonorrhoeae*
- In males, the presence of Gram negative diplococcus is diagnostic for gonorrhoea in males

CSF

- Probably will have to read a lot of these since it is a STAT test
- Recommend a 20-30 minute examination time
- Make second slide to rule out contamination
- Often bacteria are sparse in CSF
- It can be difficult to tell *Neisseria meningitidis* from *Haemophilus influenzae* if there are many WBCs and organisms with a lot of them being intracellular.
- *Listeria monocytogenes* is usually intracellular

Knowing the common causes by age group of bacterial meningitis can help

Newborn	1 – 24 months	2-6 years
Group B Strep <i>E. coli</i> K 1 <i>Listeria</i>	<i>(H. influenzae)</i> <i>S. pneumoniae</i>	<i>(H. influenzae)</i> <i>N. meningitidis</i> <i>S. pneumoniae</i>
Kids >6	Adults	Elderly
<i>N. meningitidis</i> <i>S. pneumoniae</i>	<i>S. pneumoniae</i> <i>N. meningitidis</i>	<i>S. pneumoniae</i> <i>Listeria</i>

Positive Blood Culture Bottles

- Probably the smears you will read the most
- No need to report cells or quantity of bacteria
- Usually bacteria are easy to find due to large amount present
- Must be aware that high WBC counts will cause the instruments to alarm