

Examination & Interpretation of the Gram Stained Smear

The time necessary for examination and errors in the interpretation of Gram-stained smears can be minimized by systematic examination of the slide. The preliminary evaluation proceeds as follows:

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1. First determine where the stain material is located on the slide, which side was stained, and if the smear is too thick or grossly underdecolorized.
2. Then, determine the adequacy of the specimen under low-power magnification. The hallmark of acute bacterial infection is the presence of polymorphonuclear leukocytes. Exceptions to this generalization include certain infections caused by bacteria that produce leukocyte-destroying enzymes (e.g., *Clostridium perfringens* or Group A streptococcus) or infections in neutropenic patients. The absence of granulocyte or the presence of significant numbers of epithelial or other types of cells should alert the clinician to the probability that the specimen was not adequate, and an attempt should be made to collect a better specimen. At low power, granulocyte can usually be differentiated from epithelial cells, thus enabling the examiner to focus on areas containing a predominance of inflammatory cells. This strategy is especially useful when looking at sputum, which often includes some contaminating saliva. At low power, large fungal forms (all fungi are Gram positive) can often be seen and the staining characterization of cell nuclei usually can be determined. **The oil immersion objective lens (100 x) should be used when looking for and characterizing bacteria.**
3. Evaluate the technical adequacy of the stain. It should be stressed that most errors in the interpretation of Gram-stained smears are due to errors in the preparation of the slide, such as making the smear too thick, excessive heat-fixing, and under- or overdecolorization.

The examiner may consider the smear to be adequately decolorized if Gram-positive (purple) bacteria are found next to a properly decolorized (red-counterstained) polymorphonuclear leukocyte. If the smear happens to contain only Gram-negative (red) bacteria, this guide will be lacking, and other proof is needed that the slide has not been overdecolorized. By looking at one of the thicker parts of the smear, one can often find purplish cells that have been marginally decolorized. Finding red bacteria nearby will then confirm that the organisms are Gram-negative and not simply over decolorized.

An excessively thick smear is easily recognized because it resists decolorization and microscopically shows dark masses of structures that cannot be individually distinguished. A convenient way to check staining technique, if problems are encountered, is to stain a sample of one's own mouth flora, which contains both Gram-positive and Gram-negative bacteria as well as epithelial cells.

Ordinarily, a new smear should be prepared if the original one is technically inadequate. However, if this is impossible, a badly stained smear can occasionally be salvaged by removing the immersion oil with xylol and then restaining, as follows: An underdecolorized slide is given further decolorization followed by counter staining (repeat steps 3 and 4); an overdecolorized preparation is again put through the entire Gram-stain sequence (repeat steps 1 through 4).

To obtain useful information from Gram stained preparations, you should:

Look for one or two types of bacteria near inflammatory cells: In acute infections, keep in mind that diagnostic fields usually contain only one or two types of bacteria near inflammatory cells. Exceptions to this include infections caused by leakage from heavily colonized areas, e.g., peritonitis secondary to perforated diverticulum. The temptation to interpret minor morphologic variations of a single organism as multiple types of organisms should be resisted.

Ignore contaminating flora. Varied types of bacteria in great numbers are often found near epithelial cells. These almost always are organisms that constitute the normal flora of the contaminating cell source - for instance, mouth bacteria in sputum or vaginal flora in poorly collected urine. Knowledge on the normal microbial flora in various anatomical sites may be helpful.

Beware of artifacts. Bits of irregularly shaped Gram-positive material or precipitated stain are easy to misconstrue as Gram-positive cocci. In areas with these artifacts, nearby inflammatory cells are often underdecolorized. Organisms can, however, acquire a bizarre appearance after exposure to antibiotics. Keep in mind that old, damaged, or antibiotic-treated bacteria that are normally Gram-positive may appear Gram-variable or even Gram-negative, presumably because their cell walls are more permeable to the decolorizing agent. The opposite does not hold true -- Gram-negative bacteria do not become Gram-positive in appearance with age or damage. When repeated Gram stains suggest the presence of organisms (often yeasts) that do not fit the clinical picture, consider the possibility that the Gram-stain reagents have become contaminated.

Examine the background. Small, pleomorphic Gram-negative organisms, such as *Haemophilus* or *Bacteroides*, are easily overlooked especially when Gram-positive bacteria are present. *Nocardia* and *Actinomyces* often appear as weakly Gram-positive, small, frail, branching rods that blend easily into the background. Since the *Actinomyces species* are strict anaerobes and the *Nocardia species* can be further identified by their relatively acid-fast properties, recognition of these forms on Gram-stained smear is most helpful to further identification.

Look at more than one area of the smear. Although the first bacteria seen may fit one's clinical expectation, they may not be typical of the infectious material as a whole. Check that they are indeed representative. Find more than one or two organisms before drawing any conclusion. Even when they are scarce, more than one or two examples of a bacterial type can usually be discovered in a thorough search. Making another smear is often more productive than exhaustively going over and over the original one. When no additional organisms can be detected, the significance of the limited observation should be assessed in the context of the other clinical data. In a number of difficult clinical situations, such as early or partially treated meningitis, low concentrations of organisms are not uncommon.

Don't overinterpret what is seen. The natural tendency is to leap to more specific interpretations than the Gram-stained smear warrant. To cite one pitfall, all Gram-positive cocci, including staphylococci, can occur in pairs and short chains. Thus, the impulse to conclude, for example, that all paired cocci are pneumococci can lead to clinical errors. The description should be limited to what is actually seen -- Gram-positive cocci in pairs. Then, with the recognition that morphology doesn't necessarily establish a specific etiology, the observation can be interpreted in light of the other clinical findings. This is why the physician's judgment is vital in evaluating Gram-stained smears.