GRAM STAIN

The Gram stain (which should properly be known as Gram's stain) was developed by Hans Christian Gram in 1884. This technique can distinguish between two morphologically different classes of bacteria, the so-called Gram-positives, named for their affinity for Gram's stain, and the Gram-negatives, which do not take up Gram's stain but are counterstained with safranin yielding red colored organisms.

Performance

• A thinlayer of the specimen to be analyzed is spread on a glass microscopic slide, dried, and heat fixed to the glass. Failure to use a properly dilute specimen may lead to a specimen that is too thick and is uninterpretable

• The slide is flooded with a solution of crystal violet and allowed to stain for 30 seconds to two minutes. The longer times are usually used when 'batch processing' multiple specimens.

• The crystal violet is gently rinsed off with water and the slide incubated in Gram's iodine for > 30 seconds. The iodine will fix the crystal violet to the peptidoglycans of the Gram-positive cell wall.

• After rinsing off the Gram's iodine with water, the slide is briefly decolorized with acetone. The author usually decolorizes, until there is no further runoff of crystal violet.

• The slide is then counterstained with safranin for a few seconds, rinsed and air-dried. The slide is then examined using an oil immersion lens at ~100X.

Interpretation

• In most adequate specimens, especially with purulence, sheets of polymorphonuclear leucocytes will be seen. Interspersed among them (or phagocytized by the cells) may be seen bacteria. Gram-positive (blue) spheres (cocci) in chains are consistent with streptococci whereas Gram-positive cocci in clusters are consistent with staphylococci. Even in very purulent specimens, the number of organisms seen may be surprisingly small. Gram-negative organisms may vary in length but are usually rod shaped. They may be difficult to see as the normal proteinaceous background picks up safranin and is stained red.

• It is most helpful if there is a single organism seen with consistent morphology. Observation of multiple Gram-positive and/or Gram-negative organisms speaks to colonization or contamination of the specimen.

• Occasionally, artifacts may be seen. Crystal violet may precipitate out of solution, giving the appearance of 'fungal' elements. Contaminated stain solution may give the impression that bacteria are present but a clue will be that all specimens stained with this solution will exhibit bacteria with the same stain characteristics.

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