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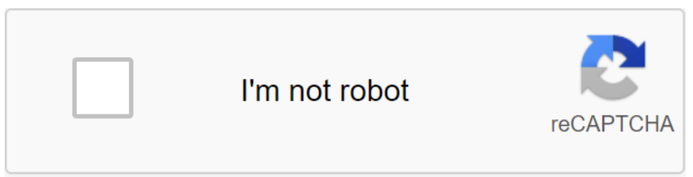

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I really have a hard time capturing Gram coloring. Can anyone help me with that? In a gram spot, one step can be omitted and still allow differentiation between gram-positive and gram-negative cells. What is this step and how will two categories of bacteria appear? -nurseinprocess-NAME (nurseinprocess - May 10, 2008, 04:27 PM) I really have a hard time capturing Gram coloring. Can anyone help me with that? In a gram spot, one step can be omitted and still allow differentiation between gram-positive and gram-negative cells. What is this step and how will two categories of bacteria appear? I wouldn't suggest omitting any step from the gram staining procedure as the whole process takes less than 5 minutes. If you need an answer for conceptual purposes for the class, I would say the initial stain with crystal purple can be omitted. By doing so, gram-positive bacteria should appear colorless/unpairenable, while a gram of negative cells will seem light pink only by taking basic fuscion or safranin counters with the last step. -phillyandrew-QUESTION (phillyandrew - May 11, 2008, 08:26 PM) THE ANSWER (nurseinprocess - May 10, 2008, 04:27 PM) I really have a hard time capturing Gram coloring. Can anyone help me with that? In a gram spot, one step can be omitted and still allow differentiation between gram-positive and gram-negative cells. What is this step and how will two categories of bacteria appear? I wouldn't suggest omitting any step from the gram staining procedure as the whole process takes less than 5 minutes. If you need an answer for conceptual purposes for the class, I would say the initial stain with crystal purple can be omitted. By doing so, gram-positive bacteria should appear colorless/unpairenable, while a gram of negative cells will seem light pink only by taking basic fuscion or safranin counters with the last step. Thank you for the answer. I read somewhere that the counter can only be one .... is that what you meant above? -nurseinprogress- (. . . . . I read somewhere that the counter can only be one .... is that what you meant above? According to the textbook chart I have, we would both be right. Dropping the final counterstain would leave a gram of negative bacteria colorless and a purple gram positive. -phillyandrew- The history of Gram Spot and how it works Gram coloring method, named after the Danish bacteriologist who originally developed it in 1882 (published in 1884), Hans Christian Gram, is one of the most important coloring techniques in microbiology. This is almost always the first test performed to detect bacteria. The main stain of the Gram method is Purple. Crystal violet is sometimes replaced by methylene blue, which is equally effective. Microorganisms that store the purple iodine complex appears purple-brown in microscopic examination. These Gram-colored microorganisms are usually classified as Gram-positive or Gram non-negative. Others that are not painted crystal purple are called Gra negative, and appear red. Gram staining is based on the ability of bacterial cell walls to hold crystal purple dye during solvent treatment. Cell walls for gram-positive microorganisms have a higher peptidoglycan and lower lipid content than a gram-negative bacterium. The walls of bacterial cells are painted crystal purple. Subsequently, iodine is added as a muzzle to form a crystalline purple iodine complex, so that the dye cannot be easily removed. This step is commonly referred to as fixing the dye. However, subsequent treatment with a decolorator, which is a mixed solvent of ethanol and acetone, dissolves the lipid layer from gram-negative cells. Removal of the lipid layer enhances the leaching of the primary stain from the cells into the surrounding solvent. In contrast, the solvent dehydrates the thicker Gram-positive cell walls, closing the pores as the cell wall shrinks during dehydration. As a result, diffusion of the purple iodine complex is blocked and the bacteria remain stained. The length of decolonization is crucial in differentiating gram-positive bacteria from gram-negative bacteria. Prolonged exposure to the decoloring agent will remove all stains from both types of bacteria. Some gram-positive bacteria can lose the stain easily and therefore appear as a mixture of gram-positive and gram-negative bacteria (Gram-variable). Finally, the main fuchsin counter is applied to the smear to give the decolored gram-negative bacteria a pink color. Some labs use safranin as a counter instead. The main fuchsin spots many gram-negative bacteria are more intense than safranin, making them easier to see. Some bacteria that are poorly colored safranin, such as hemophiline spp., Legionella spp., and some anaerobic bacteria, are easily stained with basic fuchsin, but not safranin. The polychromatic nature of the gram spot allows to determine the size and shape of both gram-negative and gram-positive bacteria. If desired, slides can be permanently installed and stored to store records. Aside from the Gram stains, there are a wide range of other coloring techniques available. Using appropriate dyes, different parts of bacteria structures such as capsules, flagella, pellets and spores can be painted. Coloring techniques are widely used to visualize components that are otherwise too difficult to see under a light microscope. In addition, special stains can be used for Other microorganisms are not easily visualized by a gram spot such as mycobacteria, rickettsia, spirochetes and others. In addition, Gram spots that allow morphological analysis of eukaryotic cells in clinical samples. Back to the front page of Gram Spot How to Make Gram Stains List of Reagents and Bunsen Burner Tools, alcohol-cleaned microscope slide, Water Reagents Crystal Violet, Gram iodine solution, acetone/ethanol (50:50 V:v), 0.1% of basic fuchsin solution Procedure A. Transmission drops suspended culture that will be reviewed on the slide with inoculation. If the culture needs to be taken from a petri dish or sloping tube culture, first add a drop or a few loops of water on the slide and aseptically transfer the minute amount of colony from the petri dish. Note that only a very small amount of culture is required; visual culture detection on the vaccination cycle already indicates that too much is accepted. When staining a clinical sample, smear a very thin layer on the slide using a wooden stick. Do not use a cotton swab, if at all possible, as cotton fibers may appear as artifacts. The smear should be thin enough to dry completely for a few seconds. The stain does not penetrate the densely used samples, which makes the interpretation very difficult. B. Spreading culture with a cycle of graft on even a thin film in a circle of 1.5 cm in diameter, about the size of a penny. Thus, a typical slide can simultaneously hold 3 to 4 small strokes if more than one crop needs to be studied. C. Air a dry culture and fix it or over a gentle flame, when moving the slide in a circular fashion to avoid localized overheating. The heat used helps cellular adhesion on a glass slide to make it possible to rinse the smear with water without significantly losing the culture. Heat can also be applied to facilitate drying of the smear. However, ring patterns can form if the heating is not homogeneous, such as taking a slide in and out of flames. A. Add a crystal purple stain over a fixed culture. Allow to stand for 10 to 60 seconds; For finely prepared slides, it is generally acceptable to pour the stain and turn off immediately. Drain the stain and gently rinse the excess stain with a stream of water from the faucet or a plastic water bottle. Note that the purpose of this step is to wash away the stain, not the fixed culture. B. Add a solution of iodine to the smear, enough to cover a fixed culture. Allow to stand for 10 to 60 seconds. Drain the iodine solution and rinse the slide with running water. Shake excess water from the surface. C. Add a few drops of the decolorizer to make the solution seep down the slide. Rinse it with water in 5 seconds. The exact time to stop is when the solvent is no longer painted as it flows through the slide. Further delay will result in excessive decoloration in the cells, and the purpose of staining will be defeated. D. Counter with a basic fuchsin solution for 40 to 60 seconds. Rinse solution with water. Spot with bead paper to remove excess water. In addition, the slide can shake to remove most of the water and dried air. It is a simple matter to prepare a control slide, bread clean wooden applicator stick and collecting small amounts of material from the interproxymal space of the teeth. This should be smeared in a drop of clean tap water on a clean glass slide. The slide can be painted as above. This material will consistently display several neutrophils and a mixture of Gram (I) and (-) organisms. The nutrophil nutrophic nuclei should be pink. Caution in the study of Gram smears is a distortion in morphology that can be caused by antimicrobial therapy. This is especially likely to happen in urine samples. Philamental and pleomorphic forms can be observed among species of gram (-) rods. The body's gram reaction can also change after antimicrobial therapy, Gram (I) bacterial can become a gram variable. Look at areas that are single cell thickness only; monitoring of thick areas will yield variable and often incorrect results. White blood cells and macrophases should color gram-negative, while sqamous epithelial cells are gram-positive. Back to the main gram spot page of the Gram spot is probably one of the most commonly used coloring procedures used in microbiology. This is one of the differential spots that are used to characterize bacteria in one of two groups: either gram-positive bacteria or gram-negative negative bacteria. Gram positive bacteria tend to have a stronger affinity for crystal purple when applying iodine grams than a gram of negative cell wall. Being a mordant, iodine gram forms a complex with crystal purple in a stain that is attached more tightly to the cell wall of gram-positive bacteria than that of a gram of negative bacteria. While gram-positive bacteria stain purple as a result of having a thick layer of peptidoglycan in the walls of their cells, a gram of negative bacteria stain red, due to the thinner pepdogtilycan layer in their cell wall (thick peptidoglycan layer allows you to keep the stain, but the thinner layer does not). TechniqueThe coloring includes 3 basic steps/processes that include:o Coloring with crystal purple (water-soluble dye) about de-coloration (using ethanol /acetone) about Counterstaining (using Safranin)Due to differences in the thickness of the peptidoglycan layer on the cell walls of these bacteria, the gramopolis bacteria will retain the crystal purple stain after the process of de-painting using ethyl alcohol/ After staining the sample with crystal purple, ethanol is used to decolorize the sample. He reaches his by dehydrating the layer of peptideglycan by tightening and reducing it. In doing so, a large crystal purple can't penetrate into the drawn layer of peptidoglycan, and therefore he he trapped in the cell wall of gram-positive bacteria. On the other hand, the outer membrane of a gram of negative cells cannot retain the crystal purple iodine complex and therefore the color is lost. Safranin is a light spot compared to crystal purple and thus it breaks the purple coloring in the gram-positive cells. TheoryIn is an aqueous solution, crystal purple divides into CV and CV-ions. These ions penetrate into the walls and membranes of both gram of positive and negative cells. CV' will interact with negatively charged components of bacterial cells, and will take purple coloring. When iodine is added, iodine cats (I- or I3-) interact with CV, leading to the formation of larger CVI complexes in the cytoplasm and outer layers of the cell. When adding a decoloring agent (ethanol) it interacts with membrane lipids as a gram of positive, and gram-negative and gram negative. This leads to the loss of the outer membrane, which in turn leaves a layer of peptideglycan exposed. For a gram of negative cells, ethanol causes the walls to be leaky and therefore they cannot hold large complexes of CV-L during de-coloration. In some coloring processes using gram stains, a picture of a gram of variables is obtained, which is a mixture of pink and purple. Some genes, such as Arthrobacter, Actinomyces and Corynebacterium, have a cell wall that is particularly sensitive to broken during cell division. This leads to a gram negative staining of a gram of positive cells. On the other hand, in the cultures of Clostridium and Bacillus, the reduced thickness of peptidalycan during growth coincides with an increase in the number of cells, which in turn stain the gram negative. Preparation1. Primary stain (crystalline purple dyes for staining) - Solution A o 2 grams of crystal purple (certified 90 percent dye content) o 20 ml of ethanol (95 percent vol/vol) - Bo solution 0.8 grams of ammonium oxalate, about 80 ml of distilled water Mix A and B to get crystal purple coloring re-ing and store for 24 hours. 2. Mordant (gram iodine) about a gram of iodine, about grams of potassium iodide, o 300 ml of distilled water, Use of solution, iodine and potassium iodide are crushed, slowly adding water with continued grinding until all iodine has completely dissolved. (Keep it in an amber bottle) 3. Decolonizing agent o ethanol, 95 percent (vol/vol)However, acetone or 1:1 acetone with ethanol, o 50 ml acetone o 50 ml ethanol (95%) 4. Counterstain (Safranin) - Working solution: o 10 ml broth solution (2.5 g Safranin O and 100 ml 95% ethanol)o 90 ml of distilled waterProcedureSmear preparation It is important to note that the thickness of the sample smear on the slide is an important factor during the slide Sample. The smear should not be too thought or too thin. Stick a slide shortcut. Bacteria - a sample smear on the slide using needle inoculation. This can also be done by injecting a drop of saline solution on a slide followed by a sample and then mixing. This should be left to dry air before fixing the heat, carefully passing the slide through the Bunsen burner (avoid burning the sample). Actinomycetes are just like bacteria, but by trying to get part of the colony on the slide while it is still intact, this can be achieved with a scalpel. Coloring Procedureo Flood slide with crystal purple staining reagent for 1 minute, about wash slide using a gentle, indirect flow of tap water for 2 seconds, flood slide with mordant (Iodine Gram), then wait a minute, about Wash slide again in a gentle, indirect stream of tap water for 2 seconds, about Flood slide with a decololoring agent then wait 15 seconds. This can also be done by adding drop by drop to slide until the decorrid agent works from the slides running clear, about the flood slide using counterstain safranin (and wait about a minute (30 seconds to 1 minute) o Wash the slide using a gentle and indirect flow of tap water to the point where the color appears in the sewage, and then a spot of dry absorbent paper, o Add a drop of dip oil to the stained sample and observe under the microscope Gramm staining helps to characterize the bacteria as a gram positive or gram negative allows microscope enthusiasts/ specialists to check the walls of the bacterial cell and membrane, which in turn affects various aspects of its pathogenicity and virulence level. : Cell Division, Cell Differentiation and Cytopathology and Learn About Microscope Coloring Kits.Return to Bacteria Front PageRetrn, to learn about the differences between Gram Positive and Gram Negative BacteriaReturn from Gram Spot to Cell P, Petrukka, A., De Mori,., Festa, A., Evangelo, B., Antinori, A., and Petrosillo, North, (2001) Community -Acquired Acinetobacter radioresist Bacteremia in HIV-positive patients. New Infectious Diseases 7 (6):1032 - 1035. G.K. Beveridge, T.J. and H.K. Clark. 1983. The chemical mechanism of the gram spot and the synthesis of a new electron-opaque marker for electron microscopy, which replaces the iodine muzzle of the spot. D. Bacteritol. 156 (2):837-845. McClelland, R., 2001. Gram is the key to microbiology. Medical laboratory observer (serial on the Internet). April 2001 6 августа 2005 г. Ссылки faculty.stcc.edu/rapp/BIOT251/gram_stain_procedure.htm 2005 г. Ссылки faculty.stcc.edu/rapp/BIOT251/gram_stain_procedure.htm which step can be omitted in gram staining

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