Compilation of microbiology staining qanda essay sample

Identification and classification of bacteria is important to make easier manipulation on the bacteria for various purposes such as for medical, research, developmental, and biotechnicalfieldsThe cell wall is the basis for classification of bacteria according to the Gram stain. Accordingto the chemical make up of bacterial cell wall, a staining procedure, Gram stain, helps usclassify bacteria into two subgroups, The cell wall can stain either positive or negative, depending on its chemistry. If the bacteria stains positive it will retain a purple/blue color. If the bacteria stains negative, the bacteria will not retain the purple/blue color, but rather have a pinkish/red color. Gram-positive bacteria have a thick layer of peptidoglycan external to the cytoplasmicmembrane. In contrast, Gram-negative bacteria have a thin layer of peptidoglycan located between the cytoplasmic membrane and a second membrane called the outer membrane. Thisregion is known as the periplasmic space. Figure 1 shows schematic representation of twotypes of bacterial cell wall structures. Other important constituents of the cell wall include thefollowing: Peptidoglycan:

This is a polymer of alternating N -acetylmuramic acid (NAM) and N-acetylglucosamine (NAG). Long strands of this alternating polymer may be linked by L-alanine, D-glutamic acid, L-lysine, D-alanine tetrapeptides to NAM . Gram-positive cellshave a much more highly cross-linked peptidoglycan structure than Gram-negative cells. Peptidoglycan is also the “ target” of antimicrobial activity. For example, penicillins interferewith the enzymes involved in biosynthesis of peptidoglycan while lysozyme physicallycleaves the NAM-NAG bond. Lipoteichoic acids:

Lipoteichoic acids (LTA) are found only in Gram-positive bacteria. These polysaccharides extend though the entire peptidoglycan layer and appear on the cellsurface. As a consequence, these structures can serve as antigenic determinants. Lipopolysaccharides:

Lipopolysaccharides (LPS) are found only in Gram-negative bacteria. These structures are composed of lipid A, which binds the LPS in the outer membrane and isitself the endotoxic portion of the molecule. The polysaccharide moiety appears on the cellsurface, serving as an antigenic determinant Periplasmic space:

The region between the peptidoglycan and LPS layers is termed the periplasmic space (coloured grey in the figure); it is a fluid or gel-like zone containing manyenzymes and nutrient-carrier proteins. Figure 1

Cell wall structures of Gram positive and Gram negative bacteriaThe Gram staining method, named after the Danish bacteriologist who originally devised it in1882 (published 1884), Hans Christian Gram, is one of the most important staining techniquesin microbiology. It is almost always the first test performed for the identification of bacteriaGram stain includes several staining and negative staining steps. The primary stain of theGram’s method is crystal violet. Gram staining is based on the ability of bacteria cell wall toretaining the crystal violet dye during solvent treatment. The cell walls for Gram-positivemicroorganisms have a higher peptidoglycan and lower lipid content than gram-negative bacteria. Bacteria cell walls are stained by the crystal violet. Iodine is subsequently added as a mordant to form the crystal violet-iodine complex so that the dye cannot be removedeasily. This step is

commonly referred to as fixing the dye. However, subsequent treatment with a decolorizer, which is a mixed solvent of ethanol and acetone, dissolves the lipid layer from the gram-negative cells.

The 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, the diffusion of the violet-iodine complex is blocked, and the bacteria remain stained. Gram-positive cells retain the crystal violet-iodine complex and thus appear purple whereas Gram-negative cells are decolorized by the alcohol or acetone treatment, but are then stained with safranin so they appear pink The length of the decolorization is critical in differentiating the gram-positive bacteria from the gram-negative bacteria. A prolonged exposure to the decolorizing agent will remove all the stain from both types of bacteria. Some Gram-positive bacteria may lose the stain easily and therefore appear as a mixture of Gram-positive and Gram-negative bacteria (Gram-variable). Finally, a counterstain of basic fuchsin is applied to the smear to give decolorized gram-negative bacteria a pink color. Some laboratories use safranin as a counterstain instead. Gram positive rod shape purple coloured Bacillus cereus is shown as positive and Gram negative rod shape, pink Pseudomonas aeruginosa bacteria shown as negative

What is Gram Staining?

Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain

procedure distinguishes between Gram positive and Gram negative groups by coloring these cells red or violet. Gram positive bacteria stain violet due to the presence of a thick layer of peptidoglycan in their cell walls, which retains the crystal violet these cells are stained with. Alternatively, Gram negative bacteria stain red, which is attributed to a thinner peptidoglycan wall, which does not retain the crystal violet during the decoloring process.

How Does Gram Staining Work?

Gram staining involves three processes: staining with a water-soluble dye called crystal violet, decolorization, and counterstaining, usually with safanin. Due to differences in the thickness of a peptidoglycan layer in the cell membrane between Gram positive and Gram negative bacteria, Gram positive bacteria (with a thicker peptidoglycan layer) retain crystal violet stain during the decolorization process, while Gram negative bacteria lose the crystal violet stain and are instead stained by the safranin in the final staining process. The process involves three steps:

1. Cells are stained with crystal violet dye. Next, a Gram’s iodine solution (iodine and potassium iodide) is added to form a complex between the crystal violet and iodine. This complex is a larger molecule than the original crystal violet stain and iodine and is insoluble in water. 2. A decolorizer such as ethyl alcohol or acetone is added to the sample, which dehydrates the peptidoglycan layer, shrinking and tightening it. The large crystal violet-iodine complex is not able to penetrate this tightened peptidoglycan layer, and is thus trapped in the cell in Gram positive bacteria. Conversely, the the outer membrane of Gram negative bacteria is degraded and the thinner peptidoglycan layer of Gram negative cells is unable to retain the crystal

violet-iodine complex and the color is lost. 3. A counterstain, such as the weakly water soluble safranin, is added to the sample, staining it red. Since the safranin is lighter than crystal violet, it does not disrupt the purple coloration in Gram positive cells. However, the decolorized Gram negative cells are stained red.

Source: http://serc. carleton. edu/microbelife/research_methods/microscopy/gramstain. html

Q: what would happen if there was a mistake when performing a gram stain and there was a reversal of crystal violet and safranin stains? You would indeed get some blue bacteria under the microscope and no pink ones, a false result if there were indeed gram negative bacteria. One of the steps in Gram's method involves washing with ethanol/acetone to remove the crystal violet from gram negative bacteria, however if the steps were reversed the safranin would be washed out of both negatives and positives and the crystal violet would stick in both cell types. Bottom line? IT WOULD NOT WORK.

Q. Both crystal violet & safranin are basic stains and may be used to do simple satains on Grampositive and? Gram negative cells. This being the case, WHAT MAKES THE GRAM STAIN A DIFFERENTIAL TEST?

It is a differential test because you can tell the difference between gram positive (that stain purple) and gram negative (that stain blue) bacteria. The answer really relates back to their cell walls. Because of the lipopolysaccharide and peptidoglycan differences in gram+ and gram- bacteria (i. e. gram-positive contain many layers of peptidoglycan with only an inner membrane and gram-neg have an outer and inner membrane that

is crosslinked by only a few layers of peptidoglycan), they react to the reagents used in the stain differently. In the first step, Crystal Violet actually stains both types purple because it enters and stains the cytoplasm. In the second step, iodine forms large crystals that are too large to escape through the cell wall.

The major difference comes in the third step with the application of acetone-alcohol. In gram-positive cells, it dehydrates the thick peptidoglycan layer and makes the cell even more impermeable to the crystal violet-iodine crystals. However, in gram-negative cells it does the opposite. Alcohol dissolves the outer membrane and leaves small holes in the peptidoglycan layer allowing the crystals to leak out of the cell. The gram-negative cells are now colorless.

In the fourth step, safranin is applied. It stains the gram negative cells red, but is too light to be seen over the crystal violet that still remains in the gram-positive cells. That is what makes a difference between the two.

Source(s):
Microbiology, an Introduction: Eighth Edition. Tortora, Funke, Case

Q. What happens if you reverse crystal violet and safranin stains in gram staining? Both bacteria types would be stained by the safranin. When the iodine is added, safranin would be “ set” in the positive. The decolorizer would wash out the safranin and then application of the crystal violet would stain the negative.

Q. What happen if you forget to add decolorizing in gram stain positive?

Staining mechanism

Gram positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of cell wall), which stains purple while Gram-negative bacteria have a thinner layer (10% of cell wall), which stains pink. Gram-negative bacteria also have an additional outer membrane which contains lipids, and is separated from the cell wall by the periplasmic space. There are four basic steps of the Gram stain, which include applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture, followed by the addition of a trapping agent (Gram's iodine), rapid decolorization with alcohol or acetone, and counterstaining with safranin or basic fuchsin.

Crystal violet (CV) dissociates in aqueous solutions into CV+ and chloride (Cl^-) ions. These ions penetrate through the cell wall and cell membrane of both Gram-positive and Gram-negative cells. The CV+ ion interacts with negatively charged components of bacterial cells and stains the cells purple.

Iodine (I^- or I_3^-) interacts with CV+ and forms large complexes of crystal violet and iodine (CV-I) within the inner and outer layers of the cell. Iodine is often referred to as a mordant, but is a trapping agent that prevents the removal of the CV-I complex and therefore color from the cell.

When a decolorizer such as alcohol or acetone is added, it interacts with the lipids of the cell membrane. A Gram-negative cell will lose its outer membrane and the lipopolysaccharide layer is left exposed. The CV-I complexes are washed from the Gram-negative cell along with the outer membrane. In contrast, a Gram-positive cell becomes dehydrated from an ethanol treatment. The large CV-I complexes become trapped within the

Gram-positive cell due to the multilayered nature of its peptidoglycan. The decolorization step is critical and must be timed correctly; the crystal violet stain will be removed from both Gram-positive and negative cells if the decolorizing agent is left on too long (a matter of seconds).

After decolorization, the Gram-positive cell remains purple and the Gram-negative cell loses its purple color. Counterstain, which is usually positively charged safranin or basic fuchsin, is applied last to give decolorized Gram-negative bacteria a pink or red color. Some bacteria, after staining with the Gram stain, yield a Gram-variable pattern: a mix of pink and purple cells are seen. The genera Actinomyces, Arthobacter, Corynebacterium, Mycobacterium, and Propionibacterium have cell walls particularly sensitive to breakage during cell division, resulting in Gram-negative staining of these Gram-positive cells. In cultures of Bacillus, Butyrivibrio, and Clostridium a decrease in peptidoglycan thickness during growth coincides with an increase in the number of cells that stain Gram-negative[10] In addition, in all bacteria stained using the Gram stain, the age of the culture may influence the results of the stain.

Q. What happen if you forget to add iodine in gram stain positive? If you do not put the iodine on a gram + stain the gram cells would get discolored by the alcohol treatment and they would be seen as gram – cells.

Q. What happens if you forget to add acetone to a gram negative stain? When performing the Gram stain, acetone is used as a wash step between the iodine and safranin. By not washing, all stains will remain crystal violet purple, and none will appear safranin red/pink.

Q. What would happen if iodine was omitted from a gram stain? Gram’s iodine is used to enhance retention to the bacterial cells. Upon using crystal violet and washing, it is vital that these cells can actually up take the CV or Safranin O that is typically used as a counterstain. One of the easiest ways to distinguish gram positive cells from gram negative cells is to look at the color. GN are pink. GP are purple. This is of course assuming that the other steps were carried out correctly and you havent over decolorized with the EtOH. Hope this helps.

Q. What is the action of iodine in grams stain?
Iodine is used to bind the Crystal Violet to the Gram Positive microbes.

Q. Why does the counterstain not change the color of all of the cells is it because the primary stain repels or masks the secondary stain? I n a gram stain the primary stain is crystal violet. Iodine then sets that dye into the gram positive cells while alcohol washes out the crystal violet from the gram negative cells. Then safranin, which is the counterstain in a gram stain, is used to dye the rest of the bacteria. This is the example I can give you of why a counterstain does not change the look in all the cells. Though safranin stains all the cells, the gram positive cells that were dyed purple from crystal violet don’t look pink – only the gram negative do.

Q. Why is it essential that the primary stain and the counterstain be of contrasting colors? It is essential that primary stain and the counterstain be of contrasting colors so that the target of the primary stain can easily be differentiated on a contrasting background.

Q. What if i ommit decolorizing, counterstain is not used, mordant was skipped in gram staining? It will be way too dark probably, and if not, everything will appear gram Positive.

Q. In gram stain, what would happen if failure to add iodine, fail to apply decolorizer, fail to apply safranin?

Gram’s iodine is a mordant. It chemically bonds the crystal violet to peptidoglycan. If you omit it, the Gram-positive cells will decolorize. If you forget to decolorize, everything will have crystal violet on it. If you don’t counterstain with safranin, It will be difficult to visualize and identify Gram-negative organisms, and their thin cell walls may have bound enough crystal violet that they will be misidentified as Gram-positive.

What is the mordant used in the gram staining procedure and what is its function? Gram’s iodine stain is applied after the culture is stained with the primary stain. It acts as a mordant, fixing the primary stain to the cell wall while lending no additional colour to the cell (i. e. the mordant itself is not a stain). The mordant is only able to fix the stain to Gram-positive bacteria because of the characteristic thick, peptidoglycan coat that they possess. Because the mordant is not able to fix the stain to Gram-negative bacteria (who’s coat have a different composition), the crystal violet stain will wash away from Gram-negative bacteria when the decolourizing agent is added.

How does a gram positive and gram negative bacteria differ in cell structure’s and How does this contribute to their differential staining properties?

Gram positive cells withold the crystal violet stain whilst gram negative cells are unable to and thus when washed with ethanol the stain washes out. This enables them to be free to take up the counterstain safranin.

How is a differential stain different from a simple stain?
A simple stain helps to visualise the structural(morphological) outlines of, say, a bacterial sample. I. e. Simple stains help to stain the outlines of bacterial cells, giving one the characteristic shape, size, and arrangments of the cells stained with the simple stain. For example, you can stain different strains of bacterial cells so that you can better visualise their overall form, size, and arrangments. However, applying a simple stain to a bacterial colony comprising of both types; cells with thick peptidoglycan layer, and cells with thin peptidoglucan layer (as an example), will not help distinguish them, and they tend to look roughly the same given that they’re both of the same strain.

To help you easily distinguish between the two different cell types within the same smear, a differential stain comes into scene. Using a differential stain, such as the basic dye; Crystal Violet, helps to firstly visualise both cell types purple. Then, by applying a “ Mordant” such as iodine solution, you help both cell types to more strongly interact with the bound purple Crystal Violet basic dye. Next, in the important step, applying a “ decolouriser” such as alcohol, helps to decolourise the bacterial cells with the thin-peptioglycan layer, whilst the cells with the thick-peptidoglycan, retain the purple stain since the weakly porous nature of the thick peptidoglycan layer do not allow much of the purple stain to get decolourise, as opposed to the relatively more porous thin peptiodoglycan layer of the other cell type. Now that we have one cell

type dyed purple, and the other decolourised, the next step is to apply a special stain called a “ counterstain” (such as Safranin) where the decolourised thin peptidoglyan-layered cells become slightly pinkish due to Safranin.

The differential staining technique illustrated above is “ Gram Stain”. And, the cells that retained the purple dye are collectively called Gram (+) cell, whilst those that became decolourised but were later pinkish due to Safranin counterstain are called Gram (-) cell. With the Gram Stain differential stain, it makes it easy for one to distinguish certain features found in one cell type but absent altogether in others.

http://www. answerbag. com/q_view/488814#ixzz1ygpUMr2T
Difference between Gram positive and gram negative bacteria.? Gram positive and gram negative refers to how a bacteria reacts to a gram stain. If it takes the initial stain, it will be purple and be considered gram positive. If it doesn’t take the initial stain, it will be pink and gram negative. The difference is the outer casing of the bacteria. A gram positive bacteria will have a thick layer of peptidoglycan (a sugar-protein shell) that the stain can penetrate. A gram negative bacteria has an outer membrane covering a thin layer of peptidoglycan on the outside. The outer membrane prevents the initial stain from penetrating.

What is the difference between a simple and a differential stain? The differential stain, such as the gram stain, stains the cell wall of gram positive bacteria, due to the high level of peptidoglycan present in the cell wall, these will be purple in color. Those that stain gram negative, do not have as much

peptidoglycan in their cell wall and will retain neglible amounts of the crystal violet and more of the saffrin. Hence those will be gram negative or red to pink. Thus this type of stain differentiates the bacteria in to two basic categories. This assists the physician in making a choice regarding antibiotics to treat the pathogen with. A simple stain just shows the basic morphology of the bacterium or bacteria present. You may wish to use bromthymol green, crystal violet… Etc. All you are looking at is morphology.

Bacterial capsule

The cell capsule is a very large structure of some prokaryotic cells, such as bacterial cells. It is a layer that lies outside the cell wall of bacteria. It is a well organized layer, not easily washed off, and it can be the cause of various diseases.

Composition

It usually consists of polysaccharides, but can be composed of other materials (e. g., polypeptide in B. anthracis). Because most capsules are water soluble[citation needed], they are difficult to stain using standard stains because most stains do not adhere to the capsule. For examination under the microscope, the bacteria and their background are stained darker than the capsule, which doesn’t stain. When viewed, bacterial cells as well as the surface they are on, are stained dark, while the capsule remains pale or colorless and appears as a ring around the cell.

Function

The capsule is considered a virulence factor because it enhances the ability of bacteria to cause disease (i. e. prevents phagocytosis). The capsule is

slippery and fragile, so when a phagocyte tries to phagocytose the bacteria, it can slip away. A capsule-specific antibody may be required for phagocytosis to occur. Capsules also contain water which protects bacteria against desiccation. They also exclude bacterial viruses and most hydrophobic toxic materials such as detergents. There are 14 different capsule types, which each impart their own specific antigenicity. Immunity to one capsule type does not result in immunity to the other types. Capsules also help cells adhere to surfaces.

Diversity

The capsule is found most commonly among Gram-negative bacteria:

Escherichia coli

Klebsiella pneumoniae

Haemophilus influenzae

Pseudomonas aeruginosa

Salmonella

However, some Gram-positive bacteria may also have a capsule: Bacillus megaterium for example, synthesizes a capsule composed of polypeptide and polysaccharides. Streptococcus pyogenes synthesizes a hyaluronic acid capsule. Streptococcus pneumoniae

Streptococcus agalactiae produces a polysaccharide capsule of nine antigenic types that all contain sialic acid (Ia, Ib, II, III, IV, V, VI, VII, VIII).

Staphylococcus epidermidis

The yeast Cryptococcus neoformans, though not a bacterium, has a similar capsule.[5] Capsules too small to be seen with an ordinary microscope, such as the M protein of Streptococcus pyogenes, are called microcapsules.

Mnemonic

A common mnemonic used to remember some encapsulated pathogens is: “ Even Some Super Killers Have Pretty Nice Capsules”

Escherichia coli, Streptococcus pneumoniae, Salmonella, Klebsiella pneumoniae, Haemophilus influenzae, Pseudomonas aeruginosa, Neisseria meningitidis, and the yeast Cryptococcus neoformans.

Demonstration of Capsule

1. India ink staining: the capsule appears as a clear halo around the bacterium as the ink can’t penetrate the capusule. 2. Serological methods: Capsular material is antigenic and can be demonstrated by mixing it with a specific anticapsular serum. When examined under the microscope, the capsule appears ‘ swollen’ due to an increase in its refractivity. This phenomenon is called as capsule swelling reaction or Quellung phenomenon. 3. Special capsule staining: These techniques employ copper salts as mordants for staining of the capsule.

Use in vaccination

Vaccination using capsular material is effective against some organisms (e. g., H. influenzae type b and S. pneumoniae).