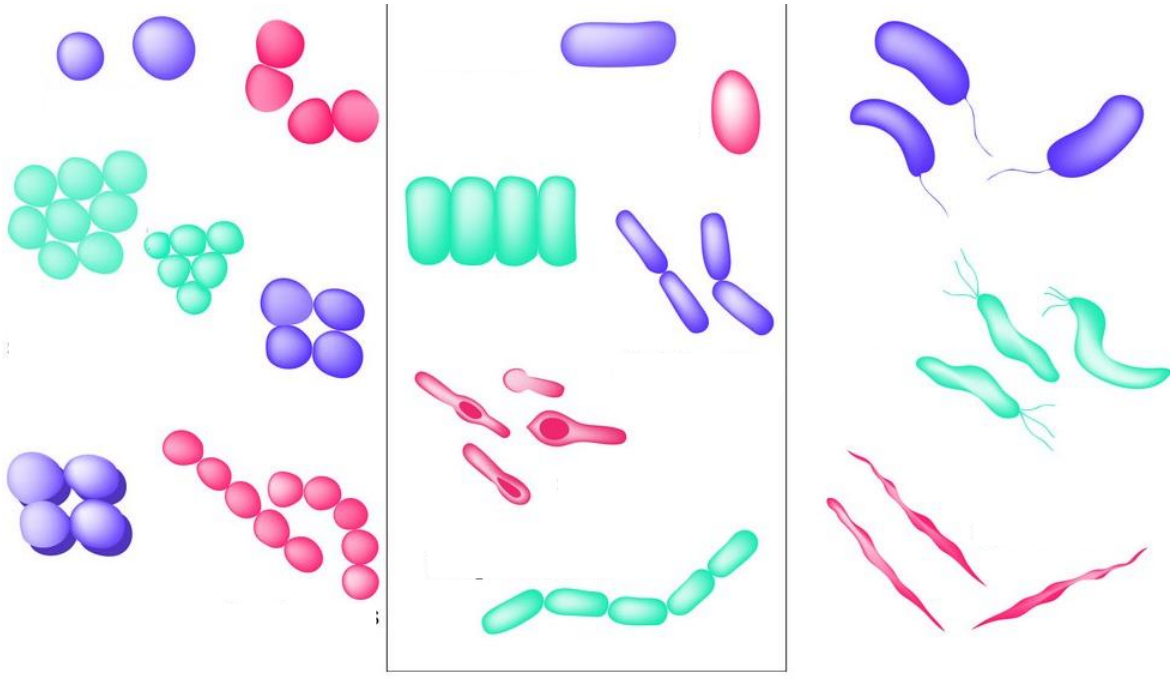
Bacterial morphology and methods of staining preparations

Part I Student completes during the lecture

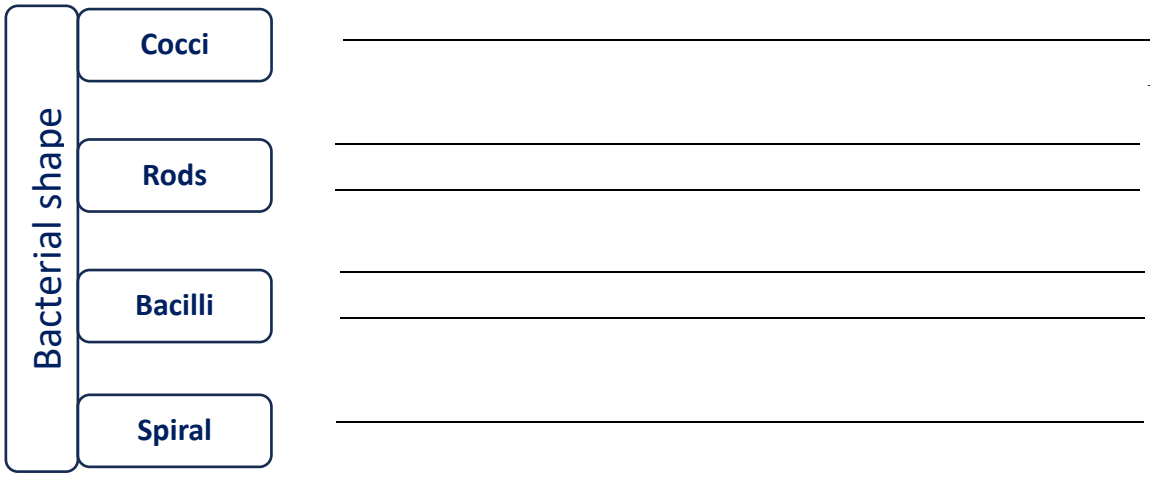
Dictionary

Pathogenicity - ..... – PATHOGEN
Virulence - ..... - VIRULENCE FACTOR
Host – .....
Routes of transmission – .....
.....
Microbial reservoir - .....
.....
Source of infection – .....
.....
Carrier - .....
.....
Endogenous vs. exogenous infection: .....
.....
Opportunistic infection: .....
.....
.....
Non-specific prevention: .....
Specific prevention: .....
Anatoxin (toxoid): .....
Anti-toxin: .....
Toxemia: .....
Antigenemia: .....
Bacteremia, viremia, fungemia: .....
Vaccine antigens: .....

Microbial morphology – provide examples of clinically important species for each group

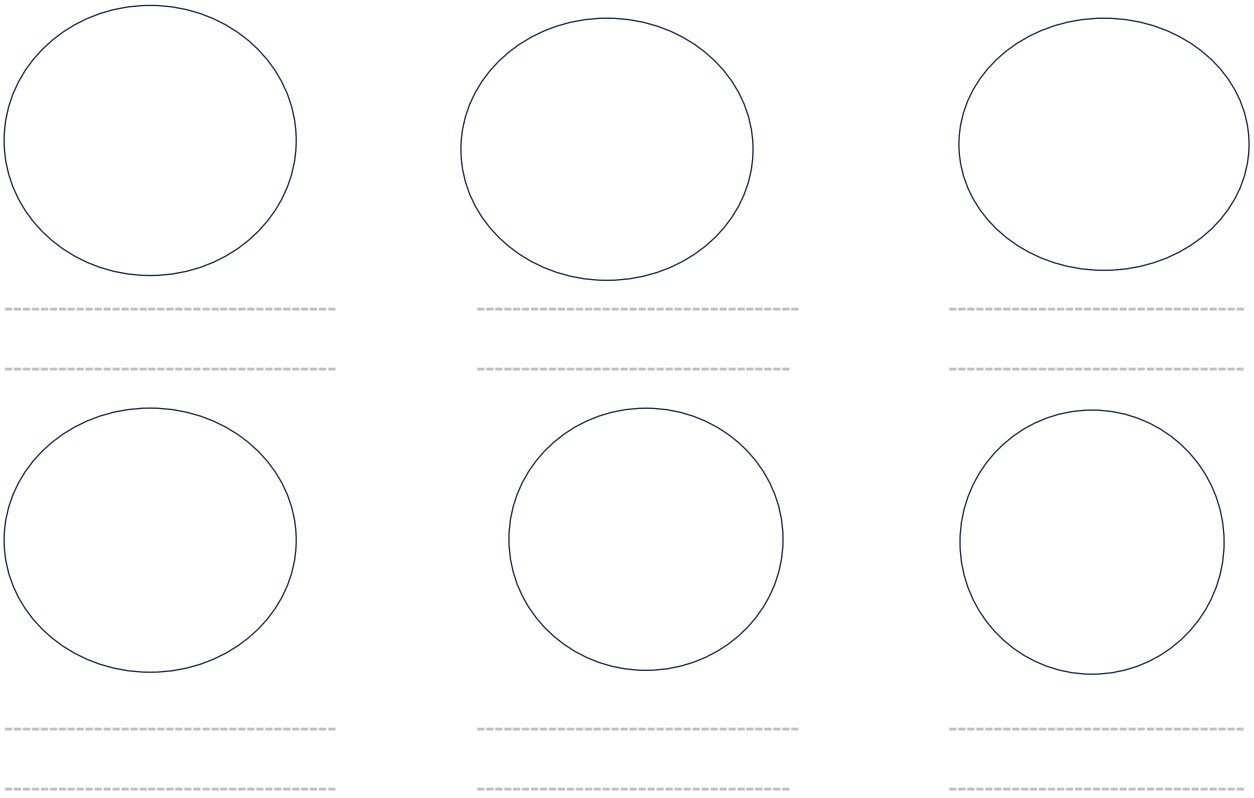


Give examples of species belonging to a given group of bacteria



Mutual arrangement of bacterial cells: clusters, clusters, chains, palisades, chains

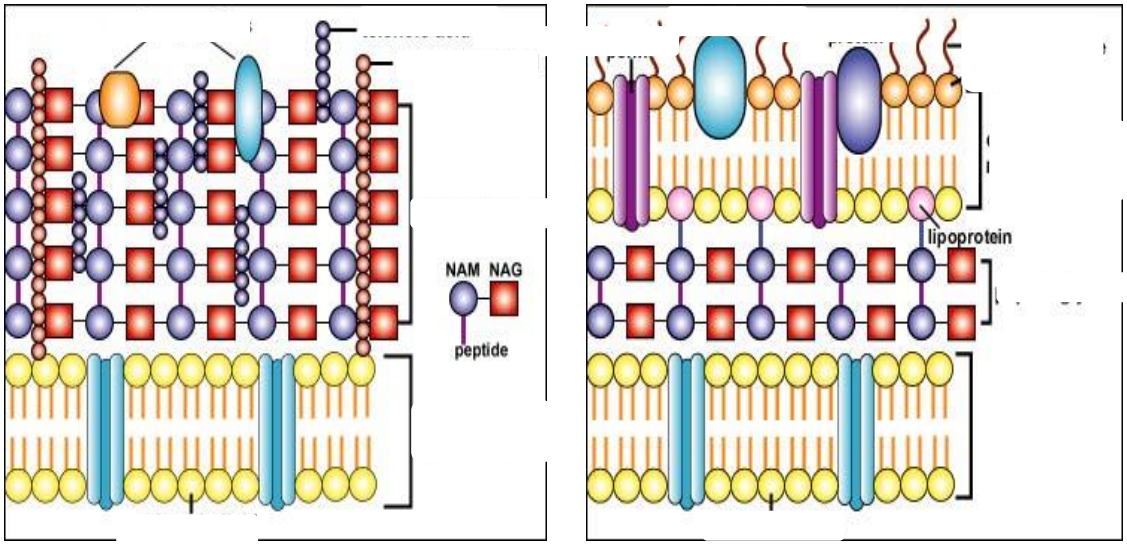
Draw (in an appropriate color) examples of GP and GN bacteria - take into account the arrangement of bacterial cells and label them with the species name



The structure of the bacterial cell wall:

Gram-positive (GP) vs Gram-negative (GN) bacteria

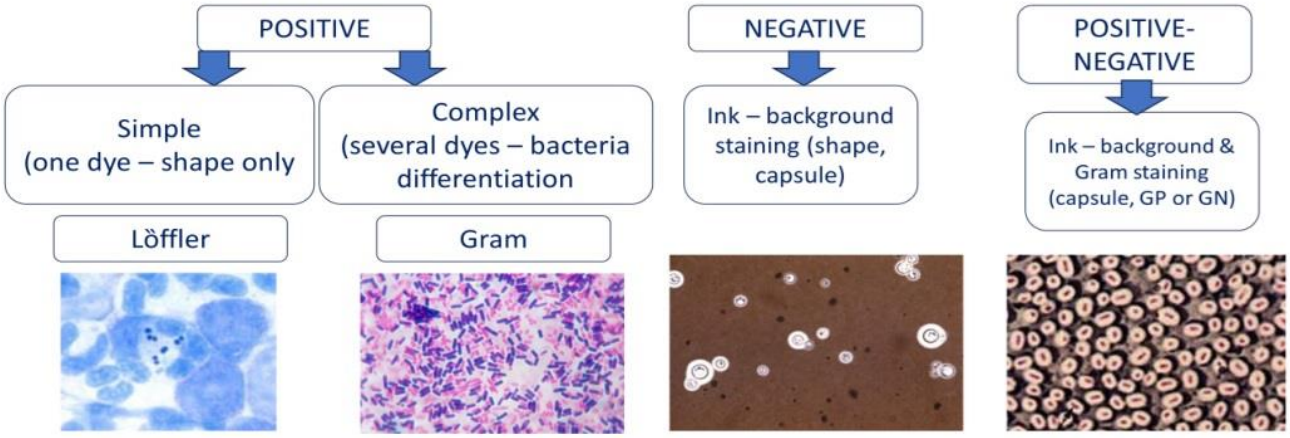
Describe the fundamental structures of a bacterial cell and determine which figure shows GP and GN bacteria



Indicate which diagram shows the GP bacterial wall and which shows the GN bacterial wall.
Describe with numbers the structure of the bacterial cell wall:
1. cytoplasmic membrane; 2, peptidoglycan; 3. LPS; 4. Lipid A; 5.LTA; 6. surface proteins; 7. phospholipids; 8. Mark where the periplasmic space is located; 9. Outer membrane; 10. Porin channel

Staining techniques

The native slide (unfixed; vital) is used to view living microorganisms under a microscope
The stained (fixed) slide enables microscopic observation of microorganisms (killed by fixing in flame or alcohol) after staining with aniline dyes



Capsule – role: .....
Glycocaliks – role: .....
Spore – role: .....
Fimbria – role: .....
LPS – role: .....
Lipoteichoic acids (LTA) – role: .....

Complex Gram staining procedure:

1. Apply a drop of sterile (sterile) saline to a glass slide (do not touch the surface of the slide with the dropper to avoid contaminating the salt in the bottle)
2. Cool the loop and stick it into the agar to cool. Mix some bacterial mass with a sterile loop in a drop of physiological saline on the slide to obtain a milky, homogeneous suspension. Then, spread the suspension over as much of the slide as possible so that it dries quickly
3. Let the preparation dry entirely at room temperature
4. Fix the preparation by pulling it through the burner flame thrice - **Use tongs!**
5. Place the fixed slide on the rack in the sink and let it cool down - **Never pour dyes onto a hot slide!**
6. Pour crystal violet over the glass for 2 min., then rinse with water and shake off the remnants of water each time so as not to dilute subsequent dyes!
7. Pour Lugol's iodine over the glass for 1 min. and then rinse with water
8. Pour alcohol over the glass for 1 min. and then rinse with water
9. Pour fuchsin over the glass for 30 seconds. and then rinse with water
10. Dry the slide gently between two sheets of tissue paper - **do not rub the paper against the surface of the slide!**
11. Apply a drop of immersion oil to the slide and examine it under a microscope at 100x magnification.

Staining procedure using the simple Lòffler method

1. Apply a drop of sterile (sterile) saline to a glass slide (do not touch the surface of the slide with the dropper so as not to contaminate the salt in the bottle)
2. Cool the loop and stick it into the agar to cool. Mix some bacterial mass with a sterile loop in a drop of physiological saline on the slide to obtain a milky, homogeneous suspension. Then, spread the suspension over as much of the slide as possible so that it dries quickly
3. Let the slide dry entirely at room temperature
4. Fix the slide thrice by pulling it through the burner flame - **Use tongs!**
5. Place the fixed slide on the rock in the sink and let it cool down - **Never pour dyes onto a hot slide!**
6. Pour Lòffler's blue over the preparation and leave for 5-10 minutes; rinse the dye with water and shake off any remaining water
7. Dry the slide gently between two sheets of tissue paper - **do not rub the paper against the surface of the slide!**
8. Apply a drop of immersion oil to the slide and examine it under a microscope at 100x magnification.

The issue to be discussed is the role of preparation in diagnostics.

The student knows/can:

1. Differences in the structure of the cell wall of GP and GN bacteria; wall antigens important in the pathomechanism of infections
2. Knows the functions/role of the fundamental structures of a bacterial cell in infections: wall, envelope, glycocalyx, cilia, fimbriae, spores.
3. Can categorize bacteria learned during classes and lectures into GP and GN, aerobic and anaerobic groups.
4. Describe the shape and arrangement of bacterial cells observed under a microscope.
5. The importance of the preparation in bacteriological diagnostics.
6. Knows the meaning of terms given in the microbiological dictionary.
7. Knows the role of microscopy in bacteriological diagnostics.

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Completion of the exercise (date and teacher's signature)