**STAINING**

1. True or **False:** It is very important to ALWAYS heat fix when performing a negative stain or a capsule stain!

Use this term bank to answer the following questions. (2,3, &4) (2 points each)

|  |  |  |
| --- | --- | --- |
| Acidic | Cells | Negative |
| Basic | Congo Red |  |
| Slide | Maneval’s |  |

1. When performing a negative stain, the acidic stain known as **\_ Congo Red** \_\_\_\_\_\_\_\_\_\_\_\_ carries a \_ **Negative** \_\_\_\_\_\_\_\_\_\_\_ charge. The stain adheres to the \_ **Slide \_\_\_\_\_\_\_** and repels the \_ **Cells** \_\_\_\_\_\_\_\_\_.
2. When performing a capsule stain, the \_ **Congo Red or Acidic**\_\_\_\_\_\_\_\_\_\_ stain adheres to the slide while the \_ **Maneval’s or Basic**\_\_\_\_\_\_\_\_\_ stain adheres to the cell.
3. The two stains used in capsule staining are **Congo Red** \_\_\_\_\_\_\_\_\_ and **\_\_\_ Maneval’s** \_\_\_\_\_\_\_.

Use this term bank to answer the following questions. (5 & 6) (2 points each)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Congo Red | Cell Wall | Endospore | Keratin | Malachite Green |
| Methylene Blue | Peptidoglycan | Negative | Vacuole | Positive |
| Sporulite |  |  |  |  |

1. A/An \_\_ **Endospore** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ is a dormant, non-reproductive structure formed by starved or stressed bacteria, typically gram \_\_ **Positive** \_\_\_\_\_\_\_ bacteria, as means for survival.
2. Due to their tough coats made up of the protein \_ **Keratin** \_\_\_\_\_\_\_\_\_\_\_\_\_\_, steam must be utilized in order for the primary stain, \_ **Malachite Green** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_, to penetrate the protein layer.

7. In a gram stain, which reagent acts as the mordant and forms a crystal violet-iodine complex?

a. Safranin b. 70% Acetone **c.** **Gram’s Iodine** d. Crystal Violet

8. What is the purpose of performing a gram stain?

a. To better visualize the flagella on bacteria

b. To stain the background and project cellular components such as a capsule

c. To visualize endospores

**d. To differentiate between bacteria with different cell wall structures**

9. When performing a gram stain from a quadrant streak isolation, a heat-fix emulsion is

required\_\_\_\_\_\_\_\_?

a. Only with gram negative bacteria

**b. Always**

c. Sometimes

d. Never

10. Circle all of the reagents that are considered to be basic stains in a gram stain?

**a. Safranin** b. Methylene Blue **c. Crystal Violet** d. Malachite green

11. What will occur if a heat-fix emulsion is performed in excess?

a. The dried smear will result in bubble formation

b**. Distortion of the cell morphology will occur**

c. The bacteria will melt off the slide when stain is applied

12. Evil scientists have genetically engineered *Bacillus anthracis* (cause of Anthrax), a known Gram positive rod, to express an additional polysaccharide-rich outer capsule with evil hopes of increasing its virulence. What effect would this have on a Gram stain of this genetically modified organism?

**a. The bacteria would still stain Gram positive.**

b. The bacteria would stain Gram negative.

c. The bacteria would stain Gram variable (some Gram positive, some Gram negative)

d. Not enough information to know!

13. Bob is performing a Gram stain on a mixed sample of Escherichia coli (Gram -) and Staphylococcus epidermidis (Gram +). While performing the Gram stain, Bob forgot to apply the counterstain, Safranin. What does Bob see under the microscope and why?

**Gram + cells will appear purple. The Gram negative cells will appear either not stained because the thin peptidoglycan layer is rinsed away OR if rinsing was weak the Gram negative cells will also appear purple.**

1. The Gram stain differentiates bacteria based on the composition of their \_\_\_\_\_\_\_\_\_\_\_\_?

|  |  |
| --- | --- |
| A. | Cell Membrane |
| B. | Nucleus |
| **C.** | **Cell Wall** |
| D. | Mitochondria |
| E. | Nuclear Membrane |

2. You are a new undergraduate researcher in a microbiology lab. You receive a sample of bacteria from your professor who asks you to record your Gram stain observations and preserve a culture for future use. Upon completion of the Gram stain, you observe purple cocci and streak a MacConkey Agar (MAC) plate to preserve the bacteria. After a 24-hour incubation period at 37o C, your professor retrieves the plate and does not see colony growth. Explain in one or two sentences the primary reason why there was no growth on the MAC plate. (2 points)

**Observed Gram Positive cocci/ MAC inhibits Gram positive growth**

3. Donald is performing a Gram stain on a mixed sample of *Escherichia coli* and *Staphylococcus saprophyticus.* While performing the Gram stain, Donald forgot to apply the counterstain, Safranin. What does Donald see under the microscope and why? (2 points)

**Purple cocci and possibly very light purple colored rods/ Decolorization step removes crystal violet from Gram negative rods and leaves the stain imbedded in Gram positive cocci. Safranin is needed to counter stain the Gram negative rods pink, since most of the crystal violet has been rinsed away.**