Difference Between Gram Stain and Acid Fast

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Key Difference – Gram Stain vs Acid Fast

Bacteria are very small microorganisms. They are transparent, and their detection is difficult under living and unstained conditions. Thus, various staining methods are developed to facilitate bacterial detection. There are three major types of staining techniques: simple staining, differential staining, and structural staining. Differential staining is a technique which uses more than one stain to differentiate bacteria. Gram stain and acid-fast stain are most popularly known as differential stains. **Gram staining is a differential staining technique, which separates bacteria into two groups known as Gram-positive bacteria and Gram-negative bacteria. The acid-fast stain is a differential stain used to identify acid-fast organisms such as Mycobacterium from non-acid fast organisms.** This is the key difference between Gram stain and Acid Fast stain.

What is Gram Stain?

Gram stain is an important differential staining technique used for bacterial identification in microbiology. This technique was introduced by Danish Bacteriologist Hans Christian Gram in 1884. Gram staining categorizes bacteria into two major groups named gram positive and gram negative, which are very important in bacterial classification and identification. Microbiologists perform gram staining as an initial step in bacterial characterization during their studies.

Bacteria are grouped based on the differences in their cell wall. Gram positive bacteria is composed of a thick peptidoglycan layer in their cell wall while gram negative bacteria is composed of a thin peptidoglycan layer in their cell wall. The outcome of the gram staining will be based on the difference in the thickness of the peptidoglycan layer of the cell wall.

Grams staining is performed using four different reagents namely; primary stain, mordant, decolorizing agent and counter stain. Crystal violet and safranin are used as the primary and counter stains, respectively while grams iodine and 95% alcohol are used as the mordant and decolourizer, respectively. The basic steps of grams stain are as follows;

1. A bacterial smear is prepared on a clean glass slide, heat fixed and cooled.
2. Smear is flooded with crystal violet for 1 – 2 minutes.
3. Smear is rinsed with slow running tap water to remove excess stains.
4. Grams iodine is applied to the smear for 1 minute.
5. Smear is rinsed with slow running tap water
6. Smear is washed with 95% alcohol for 2 – 5 seconds and rinsed with slow running tap water.
7. Smear is counter stained with safranin for 1 minute
8. Smear is rinsed with slow running tap water, dried and observed under the microscope.

At the end of the gram stain, gram negative bacteria will be observed in pink colour while grams positive bacteria will be observed in purple colour.

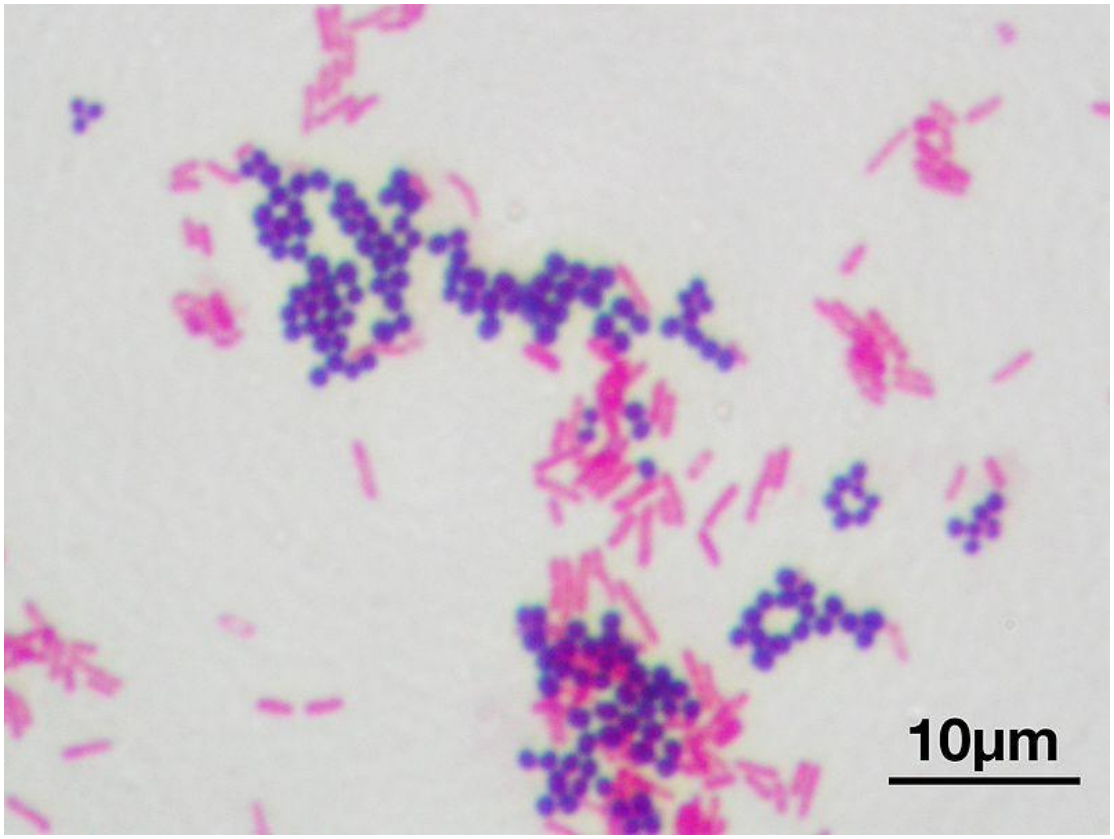

Figure 01: Gram negative and Gram positive bacteria

The outcome of the grams stain is decided by the thickness of the peptidoglycan layer in their cell wall. During the decolorizing step, primary stain and the mordant are easily removed from the gram negative bacteria and become colorless since they have a thin peptidoglycan layer. The primary stain is retained in the gram

positive bacteria since they have a thick peptidoglycan layer. The counter stain will not be effective for gram positive bacteria due to the retention of the primary stain. Thus, grams positive bacteria will be visible in primary stain colour, that is, purple colour. The counter stain will stain the gram negative bacteria, and they will be visible in pick colour, which is the safranin colour. Hence, it is easy to categorize bacteria into two groups by gram stain and it is valuable in bacterial differentiation and identification.

What is Acid Fast?

Acid-fastness is a physical property of certain bacteria, specifically their resistance to decolorization by acids during staining procedures. Once stained, these organisms resist dilute acid and or ethanol based decolorization procedures common in many staining protocols. Thus, the name 'acid fast' is given to those organisms. This property is shown due to having a high level of waxy material (mycolic acids) in their cell walls. This test is critical for the identification of *Mycobacterium tuberculosis.*

This acid fast stain was developed by Paul Ehrlich in 1882. Ehrlich's acid fast technique was modified by Ziehl-Neelsen and it is now used more frequently. Acid fast staining procedure involves three different reagents. Carbol fuschin is used as the primary stain. Acid alcohol is used as the decolourizing agent. Methylene Blue is used as the counter stain. Staining procedure is performed as follows.

1. The primary stain (carbol fuchsin) is applied to the fixed specimen on the slide (All cells will be stained in red colour).
2. The slide is heated by steaming for 5 minutes, which drives the stains into the cells properly.
3. Then the decolorizing solution is added (This removes the red dye from all cells except the acid fast bacteria).
4. Methylene blue is added as a counter stain (It colors all decolorized bacterial cells).
5. Acid fast bacteria remain red while nonacid fast bacteria stain in blue.

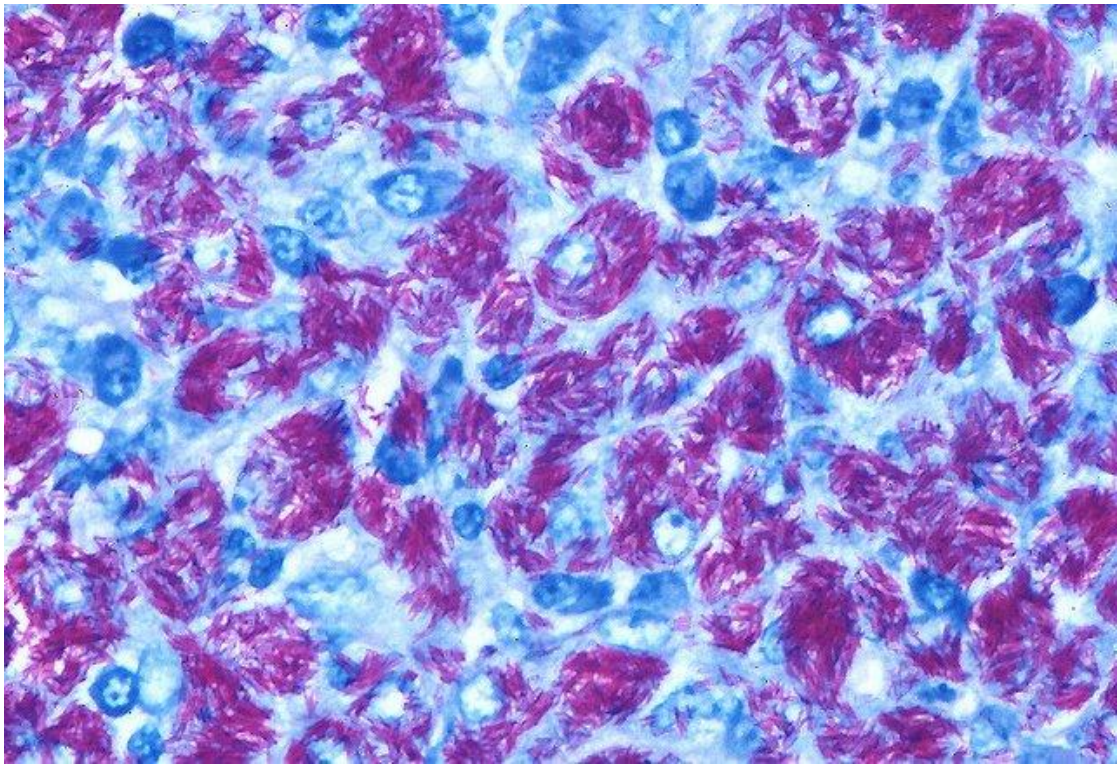

Figure 2: Acid fast mycobacteria

What are the similarities between Gram Stain and Acid Fast?

- Gram stain and Acid fast are two differential staining techniques.
- Both techniques categorize bacteria into two groups.
- Both techniques use two stains and one decolourizing.

What is the difference between Gram Stain and Acid Fast?

Gram Stain vs Acid Fast	
Gram staining is a differential staining technique, which separates bacteria into two groups Gram-positive bacteria and Gram-negative bacteria.	Acid Fast stain is a differential stain used to identify acid-fast organisms from non acid fast organisms.
Primary Stain	
Crystal violet is the commonly used primary stain in gram staining.	Carbol fuchsin is the primary stain used in acid fast.
Decolourizing Agent	

95% alcohol is used as a decolourizing agent in gram stain.	Acid alcohol is used as a decolourizing agent in acid fast.
Counter Stain	
Gram stain uses safranin as the counter stain.	Acid fast stain uses methylene blue as the counter stain.
Observation	
Gram negative bacteria are observed in pick colour and grams positive bacteria are observed in purple colour.	Acid Fast bacteria are observed in red colour and non acid fast bacteria are observed in blue colour.

Summary – Gram Stain vs Acid Fast

Visualization of microorganisms in living state is difficult. Therefore, biological stains and staining procedures are used extensively to study their properties. Differential staining is one type of staining technique used to differentiate bacteria. Gram stain and acid fast stain are two differential staining techniques. Gram staining differentiates gram negative bacteria and gram positive bacteria based on the thickness of their cell walls. Acid fast staining differentiates acid fast bacteria from non acid fast bacteria based on the mycolic acid content in the cell wall. This is the difference between acid fast and gram stain.

References:

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How to Cite this Article?

APA: Difference Between Gram Stain and Acid Fast. (2017, July 27). Retrieved (date), from http://differencebetween.com/difference-between-gram-stain-and-vs-acid-fast/

MLA: " Difference Between Gram Stain and Acid Fast" *Difference Between.Com*. 27 July 2017. Web.

Chicago: "Difference Between Gram Stain and Acid Fast". *Difference Between.Com*. http://differencebetween.com/difference-between-gram-stain-and-vs-acid-fast/accessed (accessed [date]).