**Acid Fast Staining**

Acid-fast staining was originally pioneered by a scientist named **Paul Ehrlich** in the year 1882. Later, it was modified by **Ziehl** and **Neelson** in 1883. Thus, acid-fast staining is also called Ziehl Neelson staining. It is a type of **differential staining** method used to distinguish between the acid-fast and non-acid fast bacteria.

Mycobacterium is an acid-fast bacterium, which retains the colour of carbol fuschin even after the treatment with decolourizer. The mycobacteria species retain the primary stain’s colour because they contain mycolic acid in their cell wall.

**Mycolic acid** is a waxy substance that does not allow the decolourizer to enter the cell wall due to its waxy nature. Therefore, acid-fast staining discriminates the mycobacterium species from the other groups of bacteria.

Non-acid fast bacteria quickly lose the primary stain’s colour due to the absence of mycolic acid and appears blue. In this context, we will discuss the definition, requirements, principle, process, observation and result of the acid-fast staining method.

Acid-fast staining refers to one of the [staining](https://biologyreader.com/staining.html) methods, which differentiates the Mycobacteria species from the other bacterial groups based on the **staining** **properties** and**cell wall differences**. The cell wall of Mycobacterium species contains mycolic acid, which makes it resistant towards the effect of acid decolourizer and thereby called the “**Acid-fast bacteria**”. In contrast to this, non-acid fast bacteria lack mycolic acid in their cell wall and lose the primary stain’s colour.

Red-coloured acid-fast bacteria indicates the positive result of acid-fast staining. Oppositely, blue-coloured non-acid fast bacteria indicates the negative result of acid-fast staining.

* **Examples**of**acid-fast bacteria**: Mycobacterium tuberculosis, M. leprae, M. smegmatis, M. phlei etc.
* **Examples**of **non-acid fast bacteria**: Escherichia coli, Staphylococcus aureus etc.

### **Requirements of Acid-Fast Stain**

Acid-fast staining is a differential staining procedure, which uses the combination of three reagents:

1. Ziehl Neelson Carbol fuschin
2. Acid alcohol
3. Loeffler’s Methylene blue

#### **Ziehl Neelson Carbol Fuschin (ZNCF)**

It functions as a **Primary stain**. To stain a mycobacterium, one needs a special stain like ZNCF. An ordinary stain cannot stain the mycobacterial cells. ZNCF stain has a phenolic base and a carbol fuschin dye. A **phenolic base** of ZNCF shows a high affinity towards the lipid content.

Thus, ZNCF dye can **solubilize** the lipoidal material in the cell wall and stain the cell red. Due to high lipid content, a mycobacterial cell wall is less permeable. So, a phenol base increases the **cell permeability**, by which a cell allows the stain to **penetrate**.

#### **Acid Alcohol**

It serves as a **decolourizing agent,** which contains **3% of HCL** along with **95% ethanol**. It plays an essential role in the identification of acid-fast and non-acid fast organisms. Acid-fast bacteria contains a high lipid content, which prevents the cell from binding with stains and decolourizers.

Thus, acid-fast bacteria will retain the colour of the primary stain and appear red. In contrast to this, non-acid fast bacteria lack a large amount of lipid content, as a result of which the cells lose the colour of primary stain and decolourizes.

#### **Methylene Blue**

It functions as a **counterstain** and contains 3% of methylene blue. Methylene blue stains the **decolourized cells** of non-acid fast bacteria and make them appear blue. Unlike non-acid fast, an acid-fast bacteria will not take up the colour of methylene blue and appear red.

### **Principle**

Acid-fast staining is based upon the principle of staining the bacterial cell relative to their **cell wall differences**. The cells of Mycobacteria species appear red, whereas non-acid fast bacteria appear blue.

A smear is subjected to **heat** after staining with Zeihl Neelson Carbol fuschin. During steam, carbol fuschin penetrates the bacterial cell. All the cells appear red after 2-3 minutes because a primary stain (carbol fuschin) is added at several intervals during the steaming process to prevent cell drying.

After this, a **decolourizer** (3% HCl) is added to the smear. Now, this is an important step that helps us to distinguish between the mycobacteria and the other bacteria. The mycobacteria resist the effect of decolourizer because of the substantial lipoidal material or mycolic acid in the cell wall. Thus, mycobacteria are considered **acid-fast bacteria**, as they don’t allow the penetration of the acid decolourizer into the cell, and remains appear red.

On the contrary, other bacteria will not resist the effect of decolourizer due to little or no lipoidal content. Here, the other bacteria are considered non-acid fast bacteria, as the decolourizer causes leakage of primary stain by creating pores in the cell wall. Thus, a non-acid fast cell appears colourless.

Only the decolourized or non-acid fast cells will take the blue colour of methylene blue stain on counterstaining. Conversely, the acid-fast cells will remain red in colour.

