



# Gelatin Hydrolysis Test

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# Outline

- What is a Gelatin
- Purpose of Gelatin Hydrolysis Test
- Methods for laboratory test of Gelatin
- Materials required and Procedure



# ■ Objectives

- The objective of the Gelatin Hydrolysis test is to determine the ability of bacteria to produce the enzyme gelatinase, which hydrolyzes the protein gelatin.

# ❖ What is a Gelatin?



- Gelatin refers to a **protein** derived from **collagen** that is commonly used in laboratory **culture media** for the **growth and identification of microorganisms**.
- Gelatin is a colorless and tasteless substance that is derived from the collagen found in the **connective tissues and bones** of animals.



## ■ Importance of Gelatin:

- In laboratory settings, gelatin is often incorporated into **nutrient agar or broth** to **solidify the medium** and provide a **substrate for microbial growth**.
- Gelatin is used in the **production of pharmaceuticals**, including **capsules and coatings for tablets**. It provides a **protective barrier for the active ingredients** and **facilitates their ingestion**.

- Gelatin is sometimes used in cosmetics and personal care products, such as shampoos, lotions, and face masks, for its film-forming and moisturizing properties.
- Gelatin is particularly useful in microbiology for biochemical tests such as the **gelatin hydrolysis test**, which is used to determine the ability of **microorganisms to produce gelatinases**, enzymes that hydrolyze gelatin.



# ■ What is gelatinase?

- Gelatinase is an enzyme produced by certain microorganisms, such as **bacteria**, that has the ability to hydrolyze or break down gelatin.
- Gelatinases are a type of protease, which are enzymes that catalyze the hydrolysis of peptide bonds in proteins.

- These enzymes play a role in the **degradation of extracellular proteins**, allowing microorganisms to **obtain nutrients from proteinaceous substrates**
- And **aiding in tissue invasion and pathogenesis in certain infectious diseases.**



# ❖ Gelatin Hydrolysis Test:



- Aim: Gelatin hydrolysis test is used to detect the ability of a microorganism to produce gelatinase that liquefy gelatin.
- **Principle:**
- The ability of microorganisms to hydrolyze gelatin. Some bacteria produce a proteolytic enzyme called gelatinase, that hydrolyzes gelatin.
- Most of the Enterobacteriaceae are gelatin-hydrolysis-test negative. Bacteria like Vibrio, Bacillus, and Pseudomonas are gelatin-positive.

## ❖ **Medias:**



- The Gelatin Hydrolysis test is usually performed using the following media:

1. Nutrient Agar
2. Gelatin Agar
3. Tryptic Soy Agar

- The choice of medium used in the Gelatin Hydrolysis test depends on the specific goals and objectives of the test, as well as the type of microorganisms being tested.

## ❖ Method:



- There are several methods for determining gelatinase production, all of which make use of gelatin as the substrate.
- The standard and most commonly employed method is the nutrient gelatin stab method.

## ❖ Stab method



1. Inoculate the gelatin deep with 4 to 5 drops of a 24-hour broth culture.
2. Incubate at  $35^{\circ}\text{C}$ - $37^{\circ}\text{C}$  in ambient air for up to 14 days.
  - Note: Incubate the medium at  $25^{\circ}\text{C}$  if the organism grows better at  $25^{\circ}\text{C}$  than at  $35^{\circ}\text{C}$ .
3. Alternatively, inoculate the gelatin deep from a 24-hour-old colony by stabbing four or five times, 0.5 inch into the medium.

4. Remove the gelatin tube daily from the incubator and place at 4°C to check for liquefaction.

- Note: Do not invert or tip the tube, because sometimes the only discernible liquefaction occurs at the top of the deep where inoculation occurred.

5. Refrigerate an un-inoculated control along with the inoculated tube. Liquefaction is determined only after the control has hardened (gelled).



- **Plate method:**

1. Stab-inoculate a heavy inoculum of an 18- to 24-hour-old test bacteria onto culture plates prefilled with nutrient gelatin (23 g/liter nutrient agar, 8 g/liter gelatin).
2. Incubate inoculated nutrient gelatin plates at 35°C for 24 hours.

# ❖ Result Interpretation:



- The results of the Gelatin Hydrolysis test are interpreted based on the appearance of the Nutrient Gelatin medium after incubation.
- The following are the two possible outcomes and their interpretations:
- **Positive Result:**
- If the bacteria being tested produce the enzyme gelatinase, they will hydrolyze the protein gelatin, causing the medium to become liquefied or to form a clear zone.



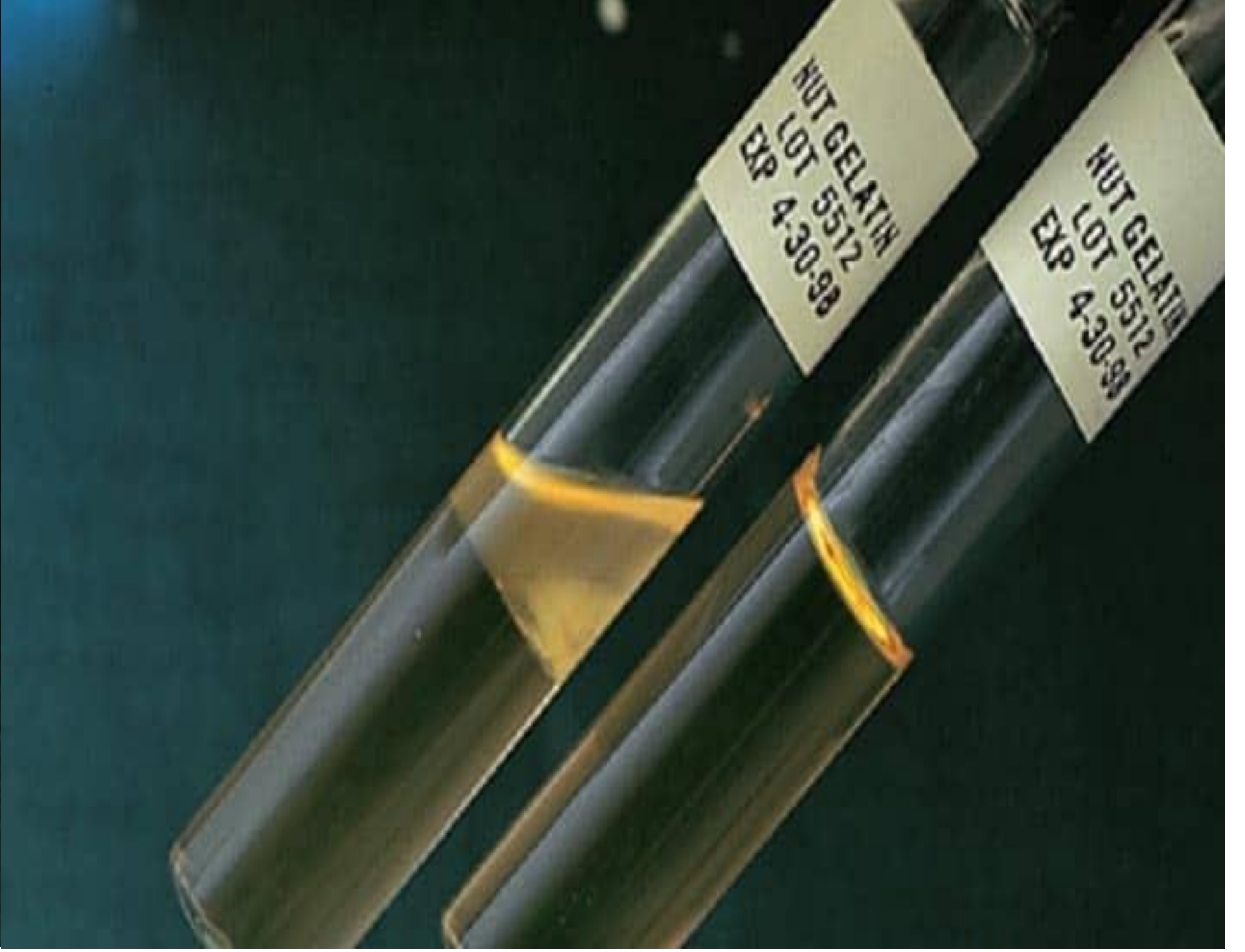
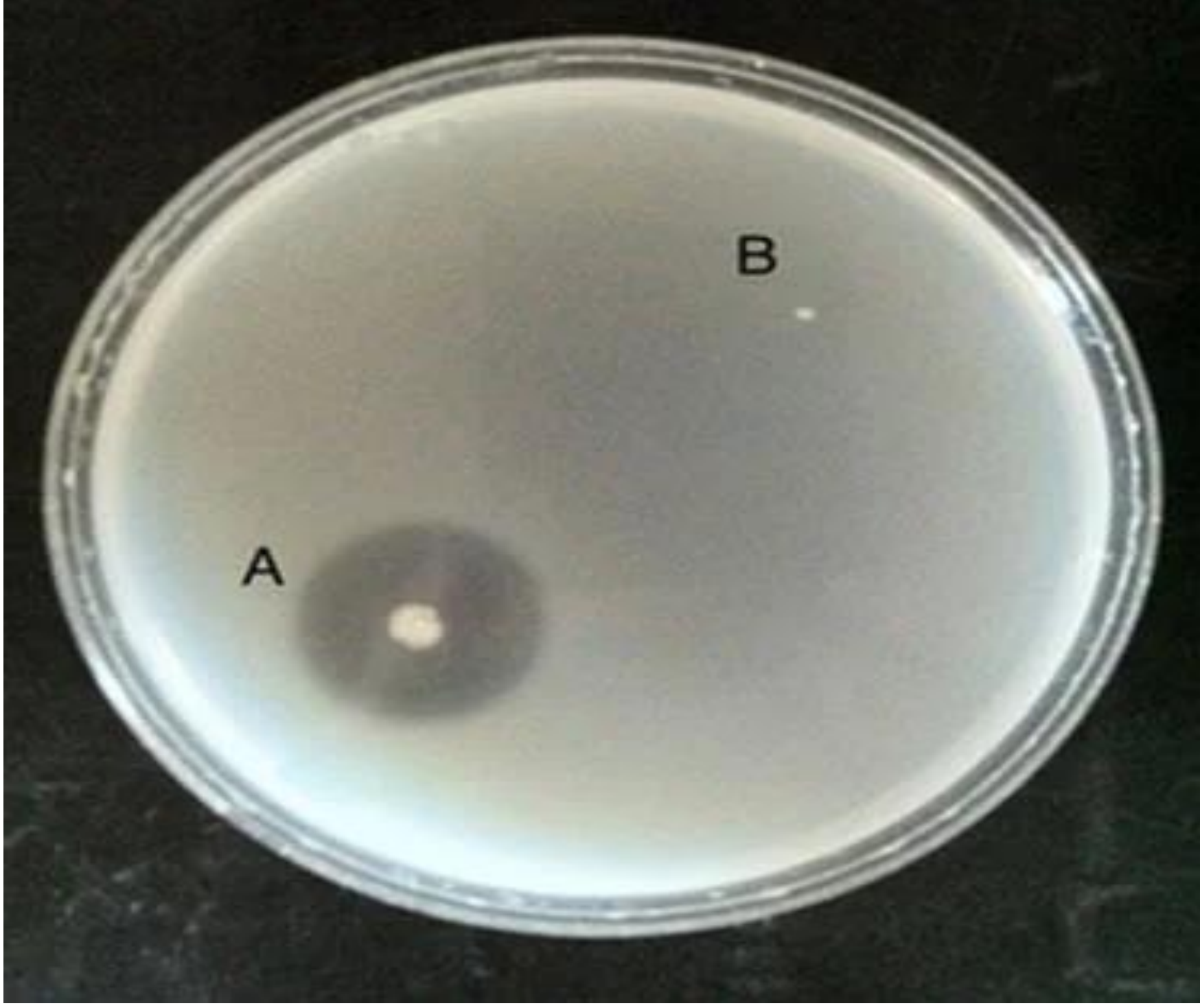
- This indicates that the bacteria are capable of producing gelatinase and that they have the ability to hydrolyze the protein gelatin. *Bacillus subtilis*.

## **2. Negative Result:**

- If the bacteria do not produce the enzyme gelatinase, the Nutrient Gelatin medium will remain solid and unchanged.
- This indicates that the bacteria do not have the ability to produce gelatinase and that they are unable to hydrolyze the protein gelatin. *Escherichia coli*



# Gelatin hydrolysis test. A, Positive B, Negative





## • **Positive Organisms:**

• These bacteria are known to produce the enzyme gelatinase and should produce a positive result in the Gelatin Hydrolysis test, indicating that they have the ability to hydrolyze the protein gelatin:

1. *Clostridium perfringens*
2. *Bacillus licheniformis*
3. *Proteus vulgaris*
4. *Streptococcus pyogenes*
5. *Staphylococcus aureus*



## • **Negative Organisms:**

• These bacteria do not produce the enzyme gelatinase and should produce a negative result in the Gelatin Hydrolysis test, indicating that they are unable to hydrolyze the protein gelatin:

1. *Escherichia coli*
2. *Klebsiella pneumoniae*
3. *Salmonella typhi*
4. *Shigella dysenteriae*
5. *Pseudomonas aeruginosa*



- **Uses :**

- This test is used to determine the ability of an organism that produce gelatinases.
- This test is helpful in identifying and differentiating species of *Serratia*, *Proteus*, *Bacillus*, *Clostridium*, *Pseudomonas* and *Flavobacterium*.
- This test differentiates pathogenic *Staphylococcus aureus* which is gelatinase-positive from non-pathogenic *epidermidis* which is gelatinase negative.



## ❖ Limitations



- Some organisms may grow poorly or not at all in this medium.
- Gelatinase usually acts at the surface of the medium. Shaking the tube while it is warm may result in false-negative interpretation.
- The Gelatin Hydrolysis test is time-consuming, requiring incubation of the bacteria and gelatin for several hours or overnight, which may not be practical for some applications.
- Gelatin Hydrolysis test requires specialized equipment and reagents, which may not be readily available or accessible in some laboratories.



**Thanks**