

# DTGH Gelatin Hydrolysis Test

## Learning Objectives

The student will

- Use aseptic techniques in the safe inoculation of various forms of media.
- Follow oral and written instructions and manage time in the lab efficiently.
- Apply correct terminology regarding microbiological techniques, instruments, microbial growth, biochemical testing, media types and forms when making observations.
- Correctly perform various inoculation techniques and describe each technique's purpose.
- Make accurate observations and appropriate interpretations of biochemical test results and use them in the identification of potentially disease causing microbes.

## Background/Theory

Gelatin is an animal protein made from collagen, a fibrous protein found in connective tissue. Microbes can utilize this protein if they can break it down into amino acids for transport into the cell. The **extracellular enzymes** that break down gelatin are called gelatinases. In bacteria, the two step process is catalyzed by two different gelatinase enzymes. Many organisms that are positive for gelatinase production are also pathogenic.

To detect the presence of gelatinases, nutrient gelatin is used. In nutrient gelatin, gelatin is the medium's solidifying agent as well as the substrate for this biochemical reaction. If the inoculated organism produces gelatinases, the protein will breakdown into smaller polypeptides and amino acids, liquefying the medium. If the organism is unable to produce gelatinases, the gelatin will remain intact and the medium solid.

To accurately interpret this test one must keep in mind that gelatin, unlike agar, will melt at temperatures above 28°C (de la Cruz, 2012). (Notice the Jell-O salad at the next picnic on a hot summer day.) For this reason, cultures will be incubated at 25°C, about room temperature. Since this is very close to 28, there is a risk that the medium will liquefy because of the temperature and not because of gelatinase activity. If the cause is temperature, the control tube will be liquid. Luckily, if gelatin is still intact, cooling the tube will allow it to solidify again. Observing the control tube first is essential here. It must be solid if accurate results in the inoculated tubes are to be obtained.

The production and action of gelatinases tends to be relatively slow often producing false negatives in this test. To minimize this, this protocol calls for a heavy inoculum from a plate culture and an incubation period of 7-14 days. Alternatively, some procedures call for incubating at 37°C to increase the enzymatic activity. Predictably, all cultures, including the sham inoculated control, will be liquid after incubation. To distinguish between liquefaction due to temperature and that due to gelatinase activity, the tubes are then cooled in an ice bath. If gelatin is still present, the medium will become solid again. If the gelatin has been hydrolyzed, the medium will remain liquid when cooled. Again, the use of a negative control for comparison is especially important, in this test.

The gelatin deeps will be inoculated with a stab inoculation technique. Follow the procedure below.

### Stab Inoculation

1. Aseptically obtain cells on the end of the inoculating needle.
2. Holding the deep tube in your non-dominant hand, remove the cap and flame the mouth of the tube as you normally do with a tube.

3. Holding the needle vertically, stab the agar straight down the center to within a quarter inch of the bottom. Then draw it straight back out of the tube. Try to follow the original stab line when removing the needle. See figure 1.
4. Again, heat the mouth of the tube after withdrawing the transfer instrument. Replace the cap and set the tube in the test tube rack.
5. Immediately incinerate the inoculating needle for a full 10 seconds before setting it down.

### Materials per student pair

2-3 gelatin deep tubes  
Inoculating needle  
Crushed ice for Lab 2

### Cultures

Fresh overnight plate cultures  
*Serratia marcescens*  
*Staphylococcus epidermidis*

### Procedure Lab 1

1. Label two of the deeps with each of the organisms. Don't forget to include the other components of the label.
2. For the third deep, label it "sham inoculation." This tube may be done for the entire class by the TA.
3. First complete the sham inoculation tube. After sterilizing your needle, aseptically perform a stab inoculation without cells. Even though this process should be sterile, be sure to incinerate the needle before the next transfer.
4. Aseptically obtain a HEAVY inoculum from a plate culture. The growth should be a visible clump on the tip of the needle. Aseptically stab inoculate the corresponding tube.
5. Aseptically inoculate the second organism in the corresponding tube in the same way.
6. Place the tubes for incubation for 7 to 14 days at 25°C.

### Procedure Lab 2

1. After incubation, check the control tube first. If it is liquid, all the tubes should be cooled by placing them in the cold box or in an ice bath until the control tube becomes solid again, about 5 minutes. Record your observations only after the control tube is solid.
2. There may only be partial liquefaction along the stab line and toward the surface of the deep where the inoculum was the heaviest. Alternatively, the entire tube may be liquid. Note the degree of liquefaction in the observations column.
3. Under result, any degree of liquefaction is a positive result. Under interpretation write "produces gelatinases" or "does not produce gelatinases."
4. When finished, place the tubes in the racks of the tube disposal tub.

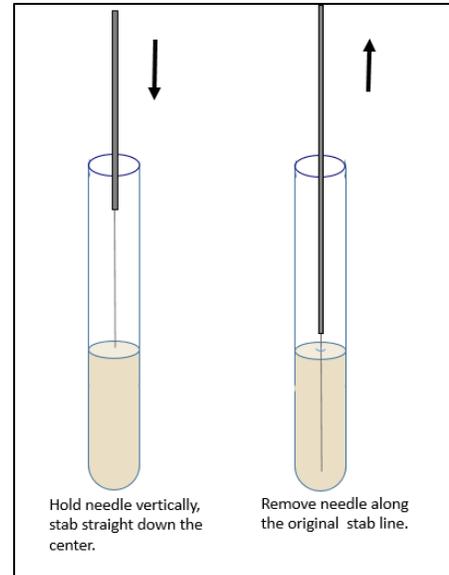


Figure 1 Stab inoculation

## Lab Report: Gelatin Hydrolysis Test

Name \_\_\_\_\_

Lab Section \_\_\_\_\_

### Data and Observations

Organism	Observations	Result +/-	Interpretation

### Post Lab Questions

- Describe how gelatinase activity could play a role in pathogenicity. (Think about the role of collagen.)
- What is the purpose of the sham inoculated tube? Does testing a sham inoculated tube prevent false positives or false negatives?
- The tubes incubate for 7-14 days because gelatinases act slowly.
  - The slow nature of the reaction may lead to false positives or false negatives?
  - Is this described as poor sensitivity or poor specificity?
  - What other test condition can you manipulate to speed up the gelatinase activity?
- Suppose you inoculated one nutrient gelatin tube with a test organism. You incubated this tube along with an uninoculated tube at 37°C. After 7 days, you remove the tubes from the incubator and both are liquid. You interpret this to mean that gelatin hydrolysis occurred in both tubes.
  - What is wrong with your interpretation?
  - If you wrote down a positive for the result, you would have a \_\_\_\_\_.

## References

de la Cruz, T. E. (2012, November). *Gelatin Hydrolysis Test Protocol*. Retrieved from American Society for Microbiology: <http://www.asmscience.org/content/education/protocol/protocol.3776>