LAB 6
HEAT RESISTANCE OF BACTERIA
AND AN INVESTIGATION INTO ENDOSPORES

Objectives

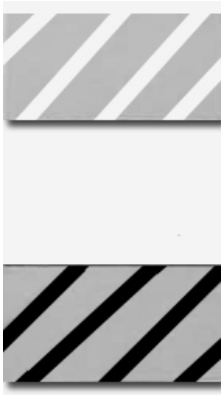
• In this lab you will learn how to:
- test the tolerance of two species of bacteria to heat
- relate heat resistance to the presence or absence of endospores
- perform an endospore stain
- distinguish endospores from vegetative bacterial cells

Introduction

All living microbes can be killed by heat. For example, Louis Pasteur developed **classic pasteurization**, in which most of the microbes in wine or milk are destroyed by exposing them to **145°F** for 30 minutes. Today, pasteurization commonly occurs at **161°F** for 15 seconds (**high-temperature, short-time pasteurization**), or **284°F** for 3 seconds (**ultra-high-temperature pasteurization**). Such **equivalent treatments** allow different combinations of temperature and time to be used to kill unwanted microbes.

However, some forms of microbes are more resistant to heat than others. For example, **vegetative cells** (living, metabolically active bacteria) are generally more vulnerable to heat than are **endospores** (dormant, protected bacterial structures). Endospores provide heat resistance in species such as *Bacillus* and *Clostridium*. Thus, it requires a higher temperature and/or longer periods of time to kill those microbes.

In laboratories and in industry, the **autoclave** is used to sterilize culture media and instruments, and to kill all microbes. Even resistant endospores are destroyed in the autoclave. Autoclaves use a combination of high-pressure steam, high temperature, and a moderate period of time (*i.e.*, 15 pounds of pressure per square inch, at **250°F**, for 15 minutes) to kill microbes. A small piece of **autoclave tape** can be used to confirm that an autoclave has reached **250°F**; it will turn black when properly heated but will remain white if that temperature is not achieved.



Autoclave tape: non-sterilized (top) and sterilized (bottom).

In contrast to what many people have heard, boiling water (**212°F**) may not be adequate to kill microbes, depending upon the microbial species and the amout of time the sample is boiled. Can endospores survive boiling? What is the lowest temperature at which microbes are killed within 10 or 30 minutes for different species of bacteria? These questions will be answered in this lab.

Part I: Heat Resistance of Bacteria

Obtain

- 14 sterile nutrient broth tubes per group
- broth cultures of *E. coli* and *Bacillis*

Procedures

Label the nutrient broth tubes #1 through #14. Use a heat-sterilized transfer loop to inoculate 7 of the broth tubes with *E. coli* (#1-7) and inoculate the other 7 tubes with *Bacillis* (#8-14). Mix the inoculum and broth thoroughly. The tubes will be assigned to the following heat treatments:

E. coli	***Bacillus***	**Heat Treatment**
#1	#8	Control (incubate at 98.6°F for 24 hours)
#2	#9	145°F for 10 minutes (then incubate as above)
#3	#10	145°F for 20 minutes (then incubate as above)
#4	#11	145°F for 30 minutes (then incubate as above)
#5	#12	212°F for 10 minutes (then incubate as above)
#6	#13	212°F for 30 minutes (then incubate as above)
#7	#14	250°F for 15 minutes (then incubate as above)

Place tubes #1 and #8 in a rack or aluminum can. These will serve as controls. Do not heat them other than placing them in the incubator (**98.6°F** for 24 hours). This rack/can will include the other tubes after they have been heated, and they will all go into the incubator at the end of the lab period.

Place tubes #7 and #14 in the blue rack at the front of the classroom. These tubes will be autoclaved together, and then placed in the incubator.

Place tubes #2, 3, 4, 9, 10, and 11 in the water bath (145°F). Allow two minutes for equilibration. Then, remove tubes #2 and #9 at 10 minutes, remove #3 and #10 at 20 minutes, and remove #4 and #11 at 30 minutes. Place them in the rack/can that will be incubated.

Place tubes #5, 6, 12, and 13 in the boiling water beaker (212°F). Allow two minutes for equilibration. Then, remove tubes #5 and #12 at 10 minutes, and remove #6 and #13 at 30 minutes. Place them in the rack/can that will be incubated.

Incubate the rack/can (containing 12 tubes) at 98.6°F for 24 hours. Observe and record the results next lab period.

Observations (next week)

Determine whether the bacteria in each tube survived. Shake each tube gently. If the broth is cloudy, then the bacteria survived and grew. If the broth is clear, then there was no growth and the heat treatment killed all vegetative cells (and, in the case of *Bacillus*, vegetative cells and endospores). Record your results in the chart below, and compare them to the other group's results.

E. coli	**Growth (+ = growth; - = no growth)**
#1	
#2	
#3	
#4	
#5	
#6	
#7	
Bacillus	
#8	
#9	
#10	
#11	
#12	
#13	
#14	

Part II: Endospore Staining

Obtain

- broth cultures of *Bacillis*

Procedures

Clean your slide well and label it. Using a sterilized transfer loop, place a loopful of *Bacillis* in the center of the circle. Immediately flame your loop again to kill the bacteria remaining on it.

Allow the small droplet containing bacteria to air dry, then heat fix the bacteria to the slide by waving the slide through the flame 3 times. You are now ready to endospore stain your sample.

Endospore Stain

Cover the air-dried, heat-fixed smear with a small piece of paper towel. Do not let any of the paper towel droop over the edge of the slide, or it will drip excess stain and/or start on fire!

Saturate the paper towl covering the sample with **malachite green**. *Without allowing the paper towel to dry out*, hold the slide over the steaming beaker of water for 5 minutes. Add more malachite green as the paper towel dries out over the steam!

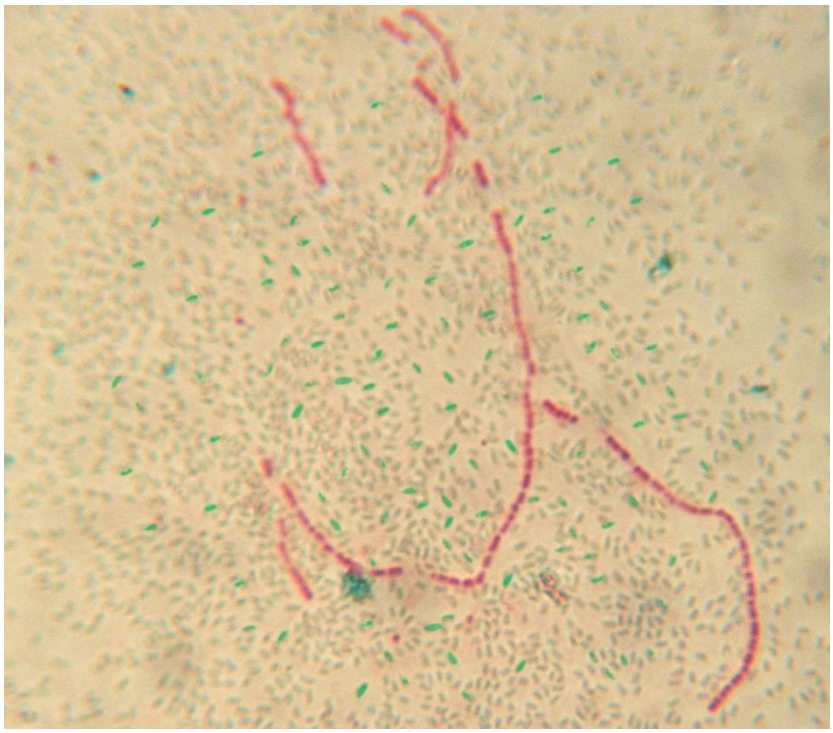
Remove the paper towel and discard it in the trash.

Immediately wash the slide with water.

Without drying, cover the smear with **safranin** for 1 minute.

Wash with tap water and allow the slide to dry.

Once the slide is completely dry, examine it under low power, then work your way up to 1000X magnification with oil immersion.



Endospore stain of *Bacillus*:
endospores appear as GREEN dots and vegetative cells will appear as PINK/RED cells.
The endospores may be inside or outside the vegetative cells.