

# Principles of sterilisation in microbiology



**ASSIGN  
BUSTER**

## Moist and dry heat

How are microorganisms destroyed by moist heat? By dry heat?

- Moist: Destroys the structure and organisms by destroying with heat. The combination of steam and pressure, the temperature is usually 121°C and 15 lbs. per inch for thirty minutes.
- Dry: Kills organisms using oxidation methods. Most resistant of the spores requires a temperature of 121°C for around two hours

Are some microorganisms more resistant to heat than others? Why?

- Endospores are extremely resistant to heat because they have a tougher coat and a low moisture content. Additionally, they have a large amount of calcium and dipicolinic acid that helps to protect them from heat.

Is moist heat more effective than dry heat? Why?

- The National Library of Medicine states, “ Moist heat penetrates faster than dry heat” (Medicine, 2015). Moist heat penetrates the molecules, additionally, the water molecules conduct heat much better than air.

Why does dry heat require higher temperatures for longer time periods to sterilize than does moist heat?

- Because water is a better conductor than air, therefore the dry heat does not penetrate as well.

What is the relationship of time to temperature in heat sterilization? Explain.

- Basically the higher the temperature the less time needed.

## The autoclave

Define the principles of sterilization with an autoclave and with a dry heat oven.

- An autoclave sterilizes using high pressurized steam heat that is made by high temperature boiling water and pressure. The steam vibrations destroy the microorganisms. Judelson states, “ Autoclaving generally involves heating in saturated steam under a pressure of approximately 15 psi, to achieve a chamber temperature of a least 121°C (250°F)— but industry, other combinations of time and temperature are sometimes used” (Judelson, 2015).
- Dry heat ovens subject the microorganisms to high temperatures and destroy the bacteria’s by drying them out and basically sterilizing them from the outside to the inside by heat and no water. Unlike an autoclave which is one piece of equipment, dry heat can include a hot air oven, incineration, radiation, microwave, Bunsen burner and flaming.

What pressure, temperature, and time are used in routine autoclaving?

- With the combination of steam and pressure, the temperature is usually at 121°C and 15 lbs. per inch for thirty minutes (Kenneth Todar, 2015).

What factors determine the time period necessary for steam-pressure sterilization? Dry-heat oven sterilization?

- The combination of temperature and time are what decides the actual time. Accordint to Mazhar Hussain, “ The thermal death time is the

time required to kill all the bacteria in a particular culture at a specified temperature. Both TDP and TDT are useful guidelines that indicate the severity of treatment required to kill a given population of bacteria” (Hussian, 2015). With dry heat the materials play an important factor.

Why is it necessary to use bacteriologic controls to monitor heat- sterilization techniques?

- They are necessary to ensure that the autoclave is working properly and efficiently. They are placed throughout the autoclave to ensure a complete testing of the autoclave.

When running an endospore control of autoclaving technique, why is one endospore preparation incubated without heating?

- The control is used to ensure that the spores are destroyed effectively and that there are no problems with the machine.

Primary media for isolation of microorganisms

Define a differential medium and discuss its purpose.

- Morello taught us that a differential media has one or more components that are used by some types of microorganisms and yet not others. (Morello, Granato, & Morton, 2013) This will allow an individual to tell the differences of a colony based on the colonies characteristics (i. e. shape, color or pattern).

Define a selective medium and describe its uses.

- Selective media chooses one type of microorganisms and then suppresses the others while not inhibiting their ability to actually grow.

MacConkey's agar would be a selective media as it selects gram negative bacteria and yet inhibits gram positive bacteria.

Why is MacConkey agar selective as well as differential?

- According to Austin Community College, “ MacConkey Agar (MAC) is a selective and differential medium designed to isolate and differentiate enterics based on their ability to ferment lactose, by using bile salts and crystal violet inhibit the growth of Gram positive organisms and lactose provides a source of fermentable carbohydrate, allowing for differentiation” (ACC, 2015).

Why is blood agar useful as a primary isolation medium?

- Blood agar would be a differential media because of its ability to destroy hemoglobin and red blood cells.

What is the major difference between Modified Thayer-Martin (MTM) and chocolate agar? When would you use MTM rather than chocolate agar?

- Modified Thayer-Martin is a selective medium that is for gram negative microorganisms that has both a medium and antibiotics, whereas chocolate agar is a non-selective medium.
- Modified Thayer-Martin is only used when the culture ordered is from the genital area or for sexually transmitted diseases, whereas chocolate is used for many different types of cultures.

Metabolic activities of bacteria

What is the color of phenol red at an acid pH?

- Yellow is the color below 7.0 pH.

What is the function of a Durham tube?

- A Durham tube contains a small inverted tube that is used for trapping the gasses that are formed when the broth inside is incubated.

(Morello, Granato, & Morton, 2013)

Why is iodine used to detect starch hydrolysis?

- When the iodine dissolves with a solution of potassium, it will turn a purple or blackish color due to the interaction with starch.

How is indole produced in SIM medium? How is it detected?

- Hardy Diagnostics states, “ The ingredients in SIM Medium enable the determination of three activities by which enteric bacteria can be differentiated; sodium thiosulfate and ferrous ammonium sulfate are indicators of hydrogen sulfide production and the ferrous ammonium sulfate reacts with H<sub>2</sub>S gas to produce ferrous sulfide, a black precipitate, additionally the casein peptone is rich in tryptophan, which is attacked by certain microorganisms resulting in the production of indole” (Diagnostics, 2015). Following the incubation period, the indole is detected with the addition of the reagents. Therefore the detection of the motility is due to the semisolid nature of the medium.

How is hydrogen sulfide demonstrated in this medium?

- The hydrogen sulfide is detected after the production of ferrous sulfide when ferrous ammonium sulfate has reacted with H<sub>2</sub>S gas.

What is the advantage of viewing mold structures in a transparent tape preparation?

- Southern University of Illinois states, “ The advantage of transparent tape preparation is it allows the fungal structures to be viewed without disrupting the characteristics of their morphology” (Illinois, 2015)

What fungus can be identified reliably by using the germ tube test?

- Morello taught us that “ Candida albicans is the most common yeast found using the germ test and is an important cause of human infection” (Morello, Granato, & Morton, 2013)

Name three stains or reagents that may be used to facilitate the microscopic detection of fungi in clinical samples.

1. Potassium hydroxide
2. Calcofluor white
3. India Ink
4. Wright, Giemsa, or Diff-Quikstains
5. Gram stain

What is the main advantage of using the slide culture technique for identifying molds?

1. It is a fast way to prepare, identify and examine fungal colonies.
2. It makes it easier to study with little disturbances.
3. According to Amrita, “ Fungi are identified mostly by close examination of its morphology and the characteristics it possess” (Amrita, 2015).

What is an opportunistic pathogen? Name three fungal specimens.

1. It is a pathogen that takes advantage of an individual that has a compromised immunity system.

## 2. Candidiasis, Aspergillosis and Cryptococcosis

### Protozoa and animal parasites

Describe the basic structures of protozoa. Can these same structures be seen in bacteria using a light microscope?

- The structure of protozoa is a single celled organisms with a true nucleus with chromosomes and surrounded by a membranous envelope. Additionally, Professor Anderson of Columbia University states, “ They also have other internal cellular structures known as organelles that perform the many physiological functions needed to maintain the life of the organism and these organelles include mitochondria that break down nutrient molecules during respiration to produce energy for the cell” (Anderson, 2015).

Are any parasitic diseases directly communicable from person to person? If so, how are they transmitted? What kinds of precautions should be taken in caring for persons with directly transmissible parasitic infections?

- Yes, but most have to have a host to be transferred. Lice for and an example is a parasite that can jump from one individual to another. Additionally, if someone is infected and does not use proper cleaning/washing of hands they can introduce a parasite through the preparation of food.
- Washing of hands and using precautionary techniques is the best way to prevent the contamination or spreading of parasites.

What parasitic forms can be seen in the feces of a patient with hookworm?



- Eggs can be seen in hookworm, Eggs and body parts can be seen in tapeworm, in cryptosporidiosis the parasite can be seen, in trichinosis, the doctor will look for the antibodies of the roundworm. (Merck, 2015)

What parasitic forms can be seen in the blood of a patient with African sleeping sickness? Filariasis? Amebiasis?

- In the blood of an individual with African sleeping sickness, the *T. b. rhodesiense* parasites can be found. In Filariasis, According to The Center for Disease Control (CDC), “ The adult worms only live in the human lymph system” (CDC, 2015). For Amebiasis The Center for Disease Control also states that, “ Diagnosis can be difficult because other parasites can look very similar to *E. histolytica* when seen under a microscope” (CDC, 2015), but the cysts can be seen in the stools/feces of an infected person.

What is meant by the “ life cycle” of a parasite? What importance does it have to those who take care of patients with parasitic diseases?

- The life cycle depends on the parasite, there are stages, the parasite infects a host, feeds off of the host and lays eggs. Sometimes the eggs hatch and sometimes they turn into cysts. The eggs, cysts and sometimes parts of the body are then transferred out of the body through fecal matter, or sometimes infected blood. When this happens they then go onto the next host and the cycle repeats.
- The main importance is protection and cleanliness. Proper use of protective wear and then washing of the hands and any surfaces that may have been contaminated are the primary rules for the containment of spreading parasites.

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