MICROBIOLOGY & PARASITOLOGY (LAB)

Plague
- A disastrous evil or affliction
- Swelling or tumors
- A deadly outbreak mentioned rats and mice
- Bubonic Plague (Yersinia pestis)

The plague of Ashdod is a highly relevant event as a historical case study of microbiological epidemic likely caused by a **microbial pathogen**. It connects to **epidemiology, zoonotic diseases**, and the **history of infectious disease**— all core aspects of **microbiology**.

Microbiology
- Field of science devoted to the study of organisms that are too small to see.
- Microorganisms are ubiquitous
- Present in soil, air, water, food, sewage, on body surfaces.

Key Important Equipments

1. *Microscope*

Purpose: Observe microorganisms down to the microscopic level.

2. *Autoclave*

Purpose: Sterilizing culture media, instruments, and decontaminating biohazardous waste
Requirement: Operates at 121C under 15 psi pressure for 15-20 minutes. (Pag under/above 121C magkakaron ng deliteration)

3. *Incubator*

Purpose: Allows for maintaining optimal temperature for microbial growth.
Usage: For bacterial and fungal culture incubation.

4. *Laminar Flow Hood*

Purpose: Handling infectious or potentially biohazardous materials (e.g, bacteria, viruses)

Working with sterile materials (e.g, cell culture media, non-pathogenic samples)

Feature: Recirculates air inwards
Allows protection of both sample and the user, as well as the environment,

Blows air toward the user
Does not protect the user and the environment

5. *Alcohol lamp and/or bunsen burner*

Purpose: Flame sterilization of inoculating loops, needles, and tubes; ensures and maintains aseptic technique.
Makes cone of protection (pushes the hot air upward kaya nagkakaron ng fire)

The Use of
Protective Personal Equipment (PPE)

A set of clothing and gear worn to protect laboratory personnel from exposure to hazardous biological agents, chemical, and physical hazards.

MICROBIOLOGY & PARASITOLOGY (LAB)

Biosafety Level

The level of containment used to prevent accidental infections or accidental environmental contamination (escape) in clinical, research, and teaching laboratories must be proportional to the biohazard potential of the organism handled in the laboratory.

BSL-1 — Not known to consistently cause disease in health adult humans, and of minimal potential hazard to laboratory personnel and the environment.
Examples: Saccharomyces cerevisiae, E. Coli K-12 and non-infectious bacteria.

BSL-2 — Moderate potential hazard to personnel and the environment. Includes bacteria and viruses that cause mild disease to humans, or are difficult to contract via aerosol in a lab setting.
Example: Hepatitis A virus, Streptococcus pyogenes, Borrelia burgdorferi (Lyme disease), Salmonella species

BSL-3 — Microbes there can either indigenous or exotic, and they can cause serious or potentially lethal disease through respiratory transmission.
Example: Yersinia pestis (plague), Mycobacterium tuberculosis, SARS, rabies virus, West Nile Virus, Hantaviruses

BSL-4 — Dangerous and exotic, posing a high risk of aerosol-transmitted infections. Infections caused by these microbes are frequently fatal and without treatment or vaccines.
Examples: Ebola virus, smallpox virus

The Aseptic Technique and Methods for Microbial Control

- **Microbicidal** (*bacteriocidal*, if bacteria). - kills microbes immediately
- **Microbistatic** (*bacterostatic*, if bacteria) - inhibits reproductive capacities of cells and maintains microbial population at a constant size.

Sterility
- A hallmark of a successful work in the microbiology laboratory

Sterilization
- Is the process of rendering a medium or material free of all forms of life.
- Correct aseptic techniques minimizes likelihood for bacterial cultures to be contaminated.
- Has **physical** and **chemical** methods for microbial control.

Chemical Methods for Control of Microbial Growth

- Antiseptics - chemical substances used on living tissue that kill or inhibit growth of vegetative microbial forms.
- Disinfectants - chemical substances that kill or inhibit the growth of vegetative microbial forms on **nonliving materials.**
- Chemotherapeutic Agents - chemical substances that destroy or inhibit growth of microorganisms that are administered **internally**.

•**Antibiotics** and **synthetic drugs**

MICROBIOLOGY & PARASITOLOGY (LAB)

Physical methods for microbial control

- The modes of action of the different chemical and physical agents of control vary, although they all produce damaging effects to one or more essential cellular structures or molecules in order to cause cell death or inhibition of growth.
- Sites of damage that can result in malfunction:
- Cell Wall
- Cell Membrane
- Cytoplasm
- Enzymes
- Nucleic Acids

Physical methods for
microbial control: temperature

- Temperature has an effect on cellular enzyme systems - marks influence on the rate of chemical reactions - life and death of microorganisms.
- High Temperature = irreversible denaturation of cellular enzymes.
- Low Temperature = inactivation of enzymes producing a static effect.

Sterilization

- Moist heat sterilization - 121C and under pressure (15psi) for 15 minutes.
- Dry heat sterilization - 160C to 180C for 1 ½ - 3 hours
- Microbes exhibit differences in their resistance to moist heat.
- Vegetative cells = 60C - 70C
- Fungi vegetative = 50C - 60C
- Bacterial spores = 100C
- Fungal spores = 70C - 80C
- **Geobacillus stearithermophilus** - used to calibrate sterilizing capacity of autoclaves
- **Endospores** - specialized heat-resistant cells that allow survival in unfavorable conditions.
- Moist heat sterilization

•**Autoclaving** - free-flowing steam effect under pressure; 121C at 15 psi for 15 minutes.
•**Tyndallization** - free-flowing steam at 100C
-Repeated procedures ensures removal of all spores and vegetative form of cells
•Pasteurization - slightly lower temperatures (<100C) at shorter intervals.

Drying Oven

- Uses high-temperature air without moisture to kill microbes through the **oxidation of cellular components**.
- It is best suited for glassware, metal surgical tools, powders, and oils that would rust or resist steam in an autoclave.
- 170C (338F): Hold for 60 minutes (or 30 minutes for uncovered sharp instruments)
- 160C (320F): Hold for 60-120 minutes
- 150C (302F): Hold for 150 minutes
- Pros: Does not rust or dull sharp metal tools, leaves no chemical residue, and safely penetrates powders and oils.
- Cons: Takes much longer than steam sterilization and will melt or burn plastics, rubber, and paper goods. Some bacterial spores can survive high temperatures.

MICROBIOLOGY & PARASITOLOGY (LAB)

Cotton Plugs

- Prevent entry of dust particles that usually contains unwanted microorganisms (i.e., aerial spores)
- Also allows air exchange maintaining aerobic conditions in cultures.
- Prevents buildup of harmful gases like ethylene.

Microbial Cultivation

- Microbes in the laboratory are grown in the laboratory using culture techniques.

Culture Media

- A solution that contains nutrients to support growth of a microorganism.

Solid

- Used to isolate colonies, study colony morphology and maintaining stock cultures.
- 1.5% - 1.8% agar

Semi-solid

- Tests for motility and can also be for fermentation studies.
- < 1.0% agar

Liquid/broth

- Used to propagate large numbers of microorganisms in fermentation studies and biochemical tests.
- No agar component

Pure Culture

- A culture that contains a single unadulterated species of cells.

Agar plates

- Provide larger surface areas for isolation and study of microorganisms
- 15 mL if plastic, 20 mL if glass

Agar slants

- Used to maintain pure cultures. Slant maximizes the surface area for microorganism growth while minimizes the medium required.
- 5 mL per tube

Deep tubes

- Used for the study of gaseous requirements of microorganisms.

Culture Media Classes

Composition	Purpose
Defined Media - exact composition amounts are determined. *Complex Media (undefined media)* - composition is unknown. *Enriched Medium* - often used to grow fastidious microorganisms.	*General Purpose* - simple media supporting a wide range of microorganisms *Selective Medium* - inhibit growth of some microorganisms *Differential Medium* - an indicator (dye) is added to determine if a chemical reaction has occurred during growth.

MICROBIOLOGY & PARASITOLOGY (LAB)

Intended Use

- The purpose of the medium

Composition

- The ingredients of the medium, usually measured per liter of distilled water.

Directions for use

- Step-by-step guide on how to prepare the agar medium correctly.