An Introduction to Microbiology

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What are microorganisms and Microbiology?

Microorganisms and Microbiology

Microorganism

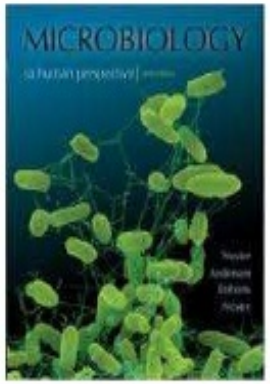
- Living things which individually are too small to be seen with the naked eye.

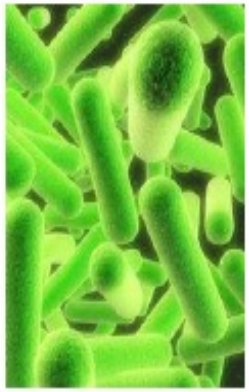
- All of the following may be considered microorganisms:
  - bacteria (eubacteria, archaebacteria)
  - fungi (yeasts, molds)
  - protozoa
  - microscopic algae
  - viruses
  - various parasitic worms

Microbiology

- Study of microorganisms
- Foundation of modern biotechnology
- Among the many specialized fields of microbiology

  -Virology, Mycology, Bacteriology, Immunology, Microbial Ecology, Biotechnological Microbiology, Environmental Microbiology, Food Microbiology, Forensic Microbiology, Molecular Biology



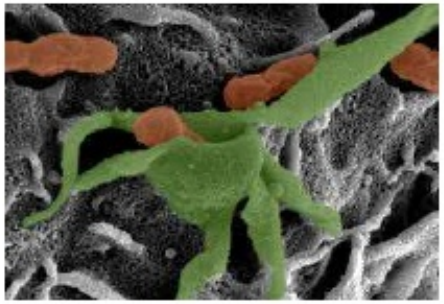


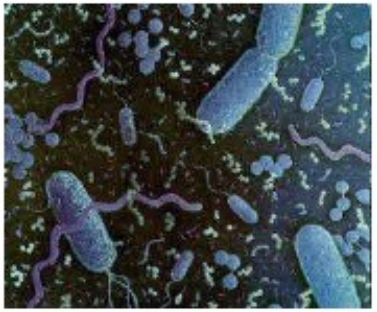
Microorganism & Microbiology cont'd

Two main themes involved in Microbiology

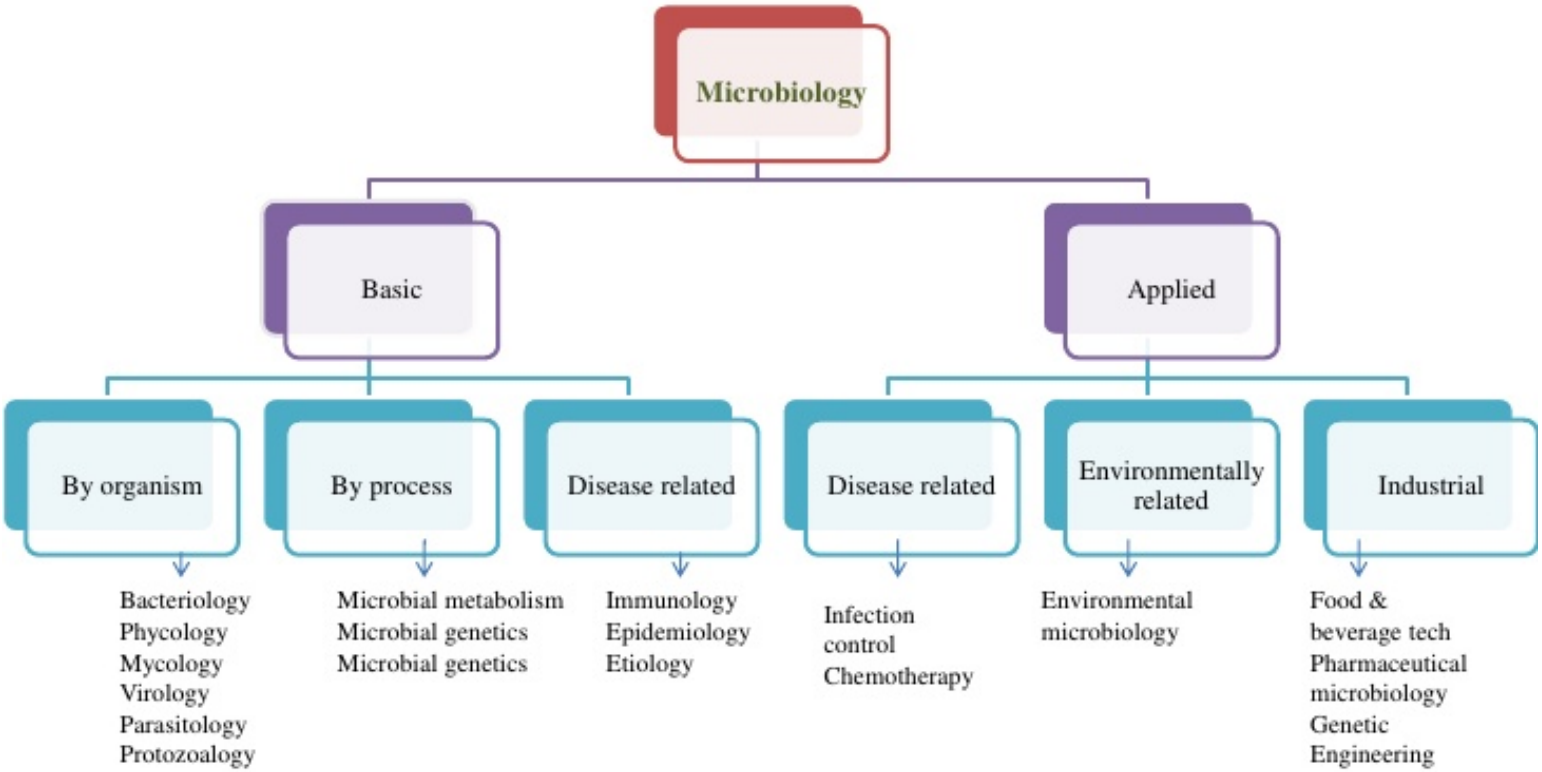
1- Basic- cellular processes

2-Applied- concerning agriculture, industry and health





Themes in Microbiology and its field



Microbes in our lives

- Some are pathogenic (disease-causing)
- Decompose organic waste
- Produces through photosynthesis (e.g.Purple sulphur bacteria must fix CO_2 to live)
- Play role in industry (e.g. fermentation to produce ethanol and acetone)
- Produce fermented food (vinegar, cheese & bread)
- Produce products used in manufacturing (cellulase) and treatment (insulin)

How do we view microorganisms?

- Units of measurement

  When talking about cells and microscopic organisms, you would be measuring using MICROMETRE (abbreviated: µ --micron) or stated as: µm (micrometer).

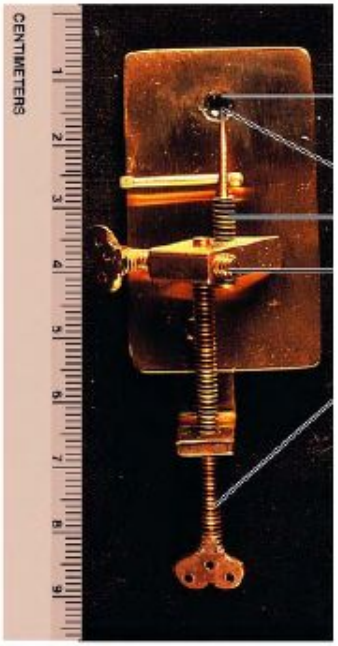
  1 µm = 1 x 10^{-6} meters/ 1 x 10^{-3} mm
  1 mm= 1 x 10^{3} nanometers/ 1 x 10^{3} µm

  To give you the idea of how small a micro metre is,
  1- a human hair is about 100 µm, wide,
  2- a red blood cell would be around 8 µm wide
  3- typical size of an animal cell would be from 10 - 100 µm

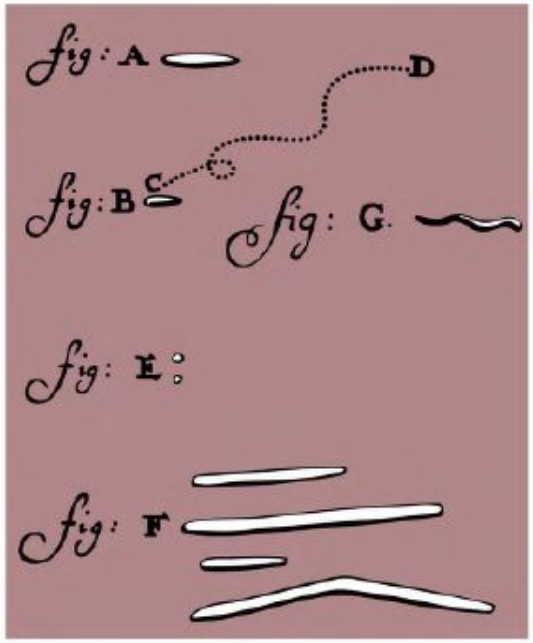
The Discovery of Microorganisms

Anton van Leeuwenhoek was the first to observe microorganisms in 1673 using his rather sophisticated (for the time) "magnifying lenses".

- **essentially began the field of microbiology**
- **the importance of microorganisms for human welfare was not appreciated until almost 200 years later!**



(b) Microscope replica

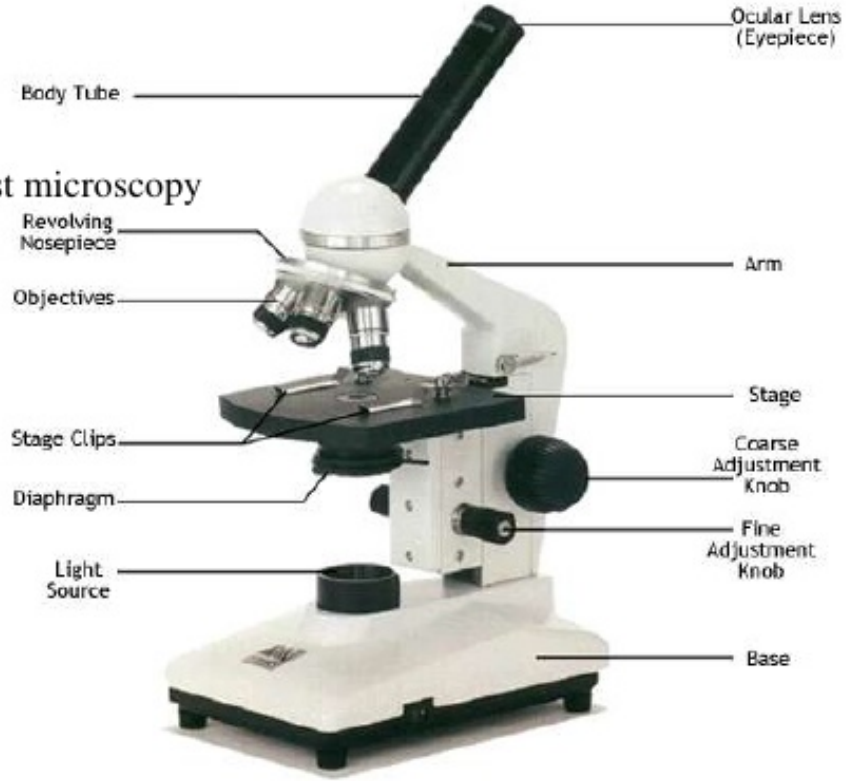


(c) Drawings of bacteria

Microscope

Light microscope

- Uses light
- Few types
  - Compound light microscopy
  - Darkfield microscopy
  - Phase-contrast microscopy
  - Differential interference contrast microscopy
  - Fluorescence microscopy
  - Confocal microscopy

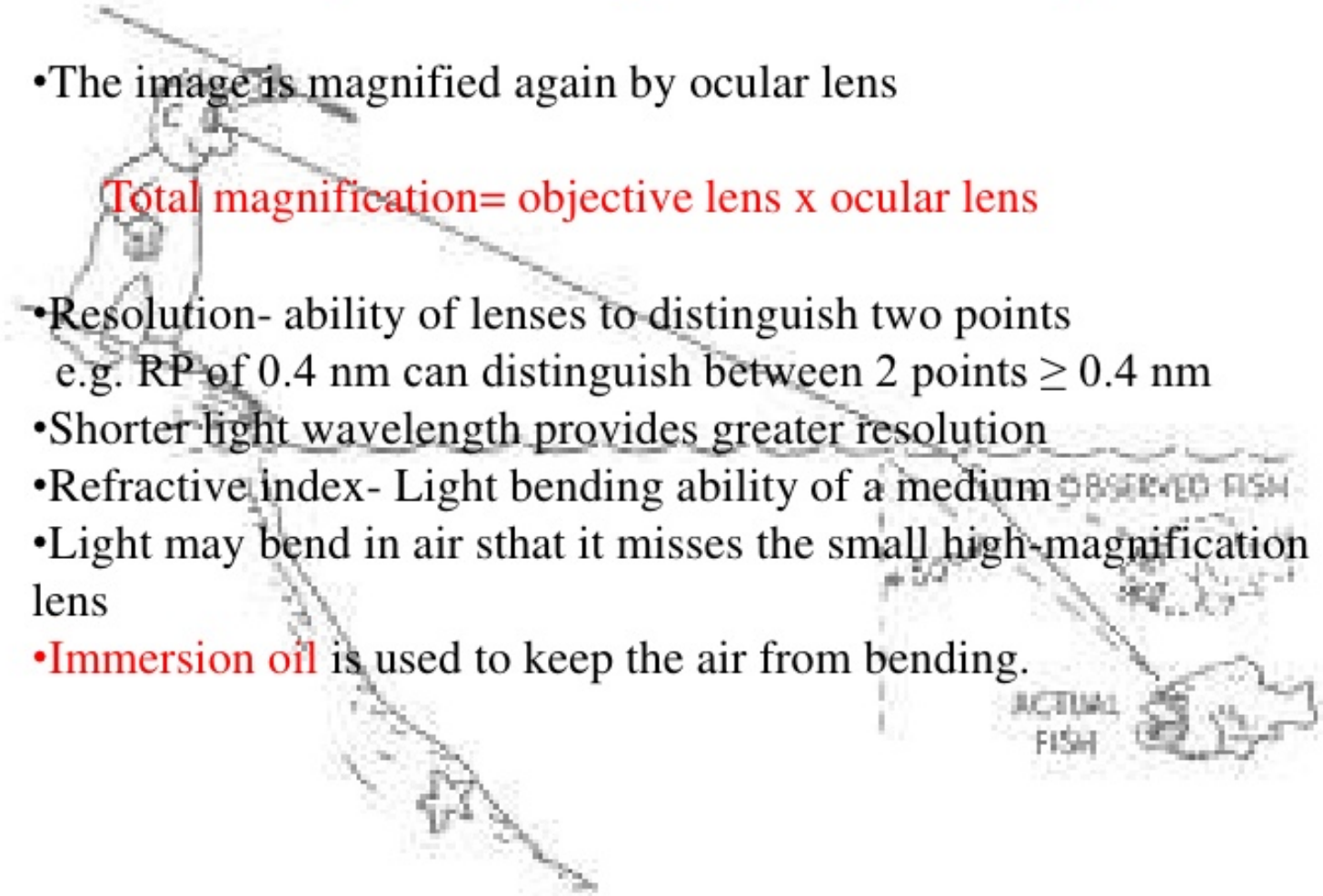


Compound light microscope

•The image is magnified again by ocular lens

Total magnification= objective lens x ocular lens

•Resolution- ability of lenses to distinguish two points
e.g. RP of 0.4 nm can distinguish between 2 points ≥ 0.4 nm
•Shorter light wavelength provides greater resolution
•Refractive index- Light bending ability of a medium
•Light may bend in air sthat it misses the small high-magnification lens
•Immersion oil is used to keep the air from bending.



Compound Microscope

https://youtu.be/4x-2GHBel0A

Types of Microscopes

Light Microscope - found in most schools, use compound lenses and light to magnify objects. The lenses bend or refract the light, which makes the object beneath them appear closer.

Stereoscope - this microscope allows for binocular (two eyes) viewing of larger specimens. (The spinning microscope at the top of this page is a stereoscope)

Scanning Electron Microscope - allow scientists to view a universe too small to be seen with a light microscope. SEMs do not use light waves; they use electrons (negatively charged electrical particles) to magnify objects up to two million times.

Transmission Electron Microscope - also uses electrons, but instead of scanning the surface (as with SEM's) electrons are passed through very thin specimens. Specimens may be stained with heavy metal salts

Development of microscopy

•(384-322) Aristotle and others believed that living organisms could develop from non-living materials.
•1590: Hans and Zacharias Janssen (Dutch lens grinders) mounted two lenses in a tube to produce the first compound microscope.
•1660: Robert Hooke (1635-1703) published "Micrographia"; drawings and detailed observations of biological materials made with the best compound microscope and illumination system of the time.
•1676: Anton van Leeuwenhoek (1632-1723) 1st person to observe microorganisms.
•1883: Carl Zeiss and Ernst Abbe pioneered developments in microscopy (such as immersion lenses and apochromatic lenses which reduce chromatic aberration) exist until the present day.
•1931: Ernst Ruska constructed the 1st electron microscope.

The Golden Age of Microbiology

Many landmark discoveries in microbiology occurred in the last half of the 19th century:

- the first vaccine (cowpox lesions to prevent smallpox)
  - *Edward Jenner* (1789)
- importance of aseptic techniques in hospitals
  - *Ignaz Semmelweis* (1848) – hand washing
  - *Florence Nightingale* (1854) – general cleanliness
  - *Joseph Lister* (~1860) – use of surgical antiseptics
- the first epidemiological study (identifying the source of a cholera outbreak)
  - *John Snow* (1854)

Contributions of *Louis Pasteur*

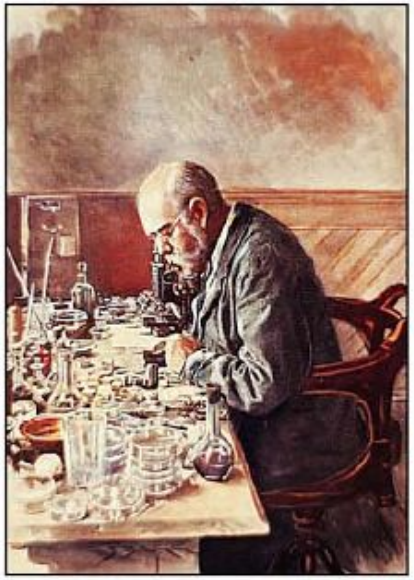
- proposed "Germ Theory" of disease (1857)
- disproved concept of *spontaneous generation* (1861)
  - i.e., microbes do NOT arise from non-living material

Louis Pasteur (1822–1895)

- showed fermentation to be carried out by microbes (1861)
- developed technique of pasteurization
- developed several *attenuated* vaccines

Contributions of *Robert Koch*

- identified the first bacterial pathogens:
  - *Bacillus anthracis* (anthrax – 1876)
  - *Mycobacterium tuberculosis* (tuberculosis – 1882)
- proposed method to identify the microbial agent responsible for a given disease (Koch's Postulates)
- developed numerous advances in microbiological techniques:
  - simple staining methods
  - fixation of specimens to slides
  - use of solid growth media
  - pure culture techniques
  - methods for counting microbes



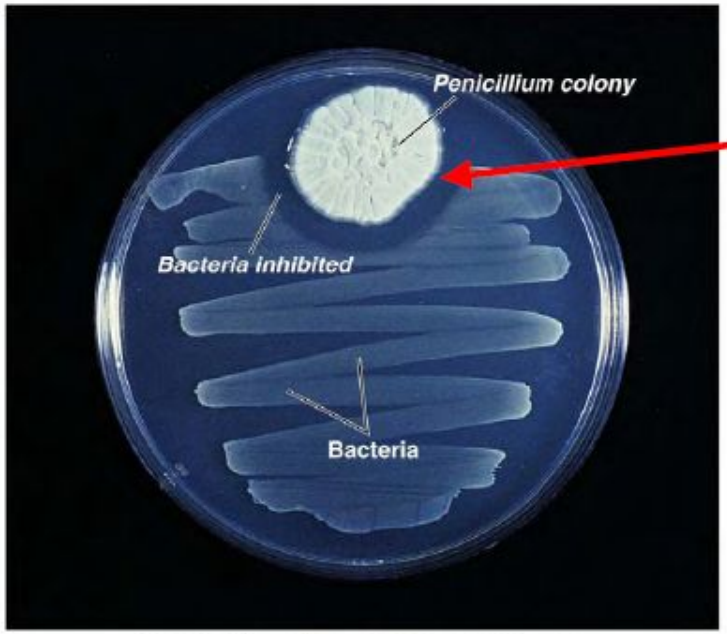
Robert Koch (1843–1910)

Penicillin Antibiotic

Sir Alexander Fleming

Other Landmarks in Microbiology

- first evidence of viruses (tobacco mosaic virus)
  - *Dmitri Ivanowski* (1892)
- the first synthetic antimicrobial chemicals
  - *Paul Erlich* (1908)



- discovery of the first antibiotic (penicillin)
  - *Alexander Fleming* (1928)
- discovery of prions
  - *Stanley Prusiner* (1997)

Culturing Microorganisms

Methods of Culturing Microorganisms: The Five I's

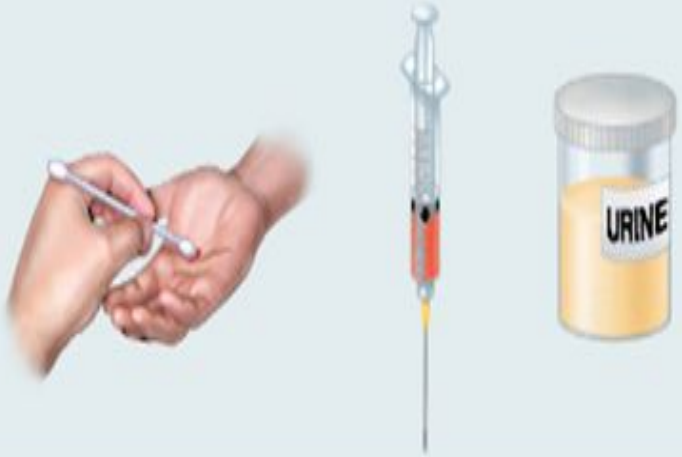
Microbiologists use five basic techniques to manipulate, grow, examine, and characterize microorganisms in the laboratory:

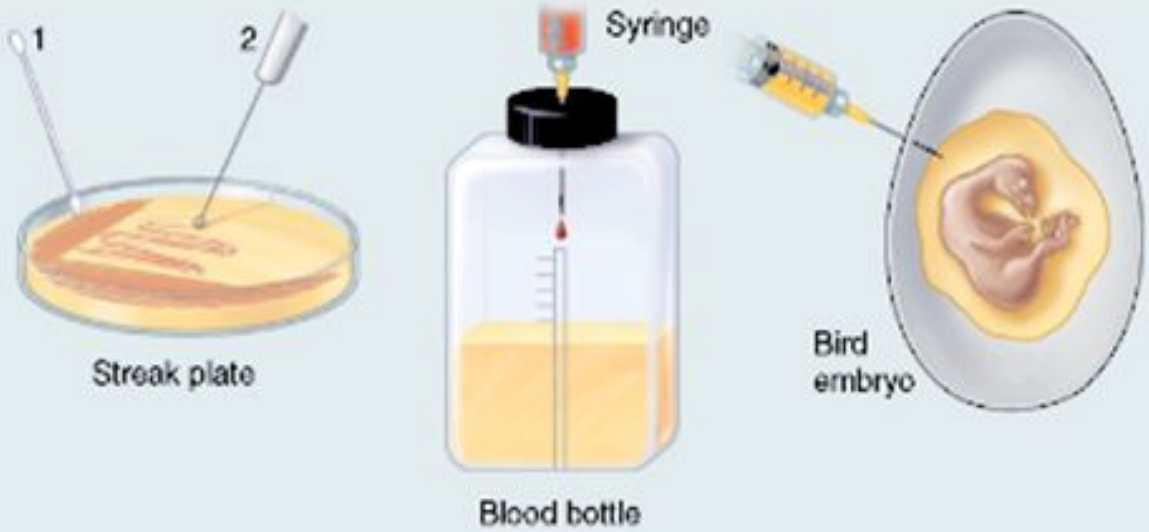
Inoculation, Incubation,

Isolation, Inspection, and

Identification

Specimen Collection:
Nearly any object or material can serve as a source of microbes. Common ones are body fluids and tissues, foods, water, or soil. Specimens are removed by some form of sampling device: a swab, syringe, or a special transport system that holds, maintains, and preserves the microbes in the sample.

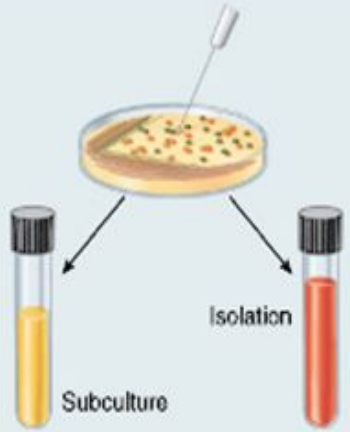




1. Inoculation:
The sample is placed into a container of sterile **medium** containing appropriate nutrients to sustain growth. Inoculation involves spreading the sample on the surface of a solid medium or introducing the sample into a flask or tube. Selection of media with specialized functions can improve later steps of isolation and identification. Some microbes may require a live organism (animal, egg) as the growth medium.

Incubator

2. Incubation:
An incubator creates the proper growth temperature and other conditions. This promotes multiplication of the microbes over a period of hours, days, and even weeks. Incubation produces a culture—the visible growth of the microbe in or on the medium.



3. Isolation:
One result of inoculation and incubation is **isolation** of the microbe. Isolated microbes may take the form of separate colonies (discrete mounds of cells) on solid media, or turbidity (free-floating cells) in broths. Further isolation by subculturing involves taking a bit of growth from an isolated colony and inoculating a separate medium. This is one way to make a pure culture that contains only a single species of microbe.

4. Inspection:
The colonies or broth cultures are observed macroscopically for growth characteristics (color, texture, size) that could be useful in analyzing the specimen contents. Slides are made to assess microscopic details such as cell shape, size, and motility. Staining techniques may be used to gather specific information on microscopic morphology.

5. Identification:
A major purpose of the Five I's is to determine the type of microbe, usually to the level of species. Information used in identification can include relevant data already taken during initial inspection and additional tests that further describe and differentiate the microbes. Specialized tests include biochemical tests to determine metabolic activities specific to the microbe, immunologic tests, and genetic analysis.

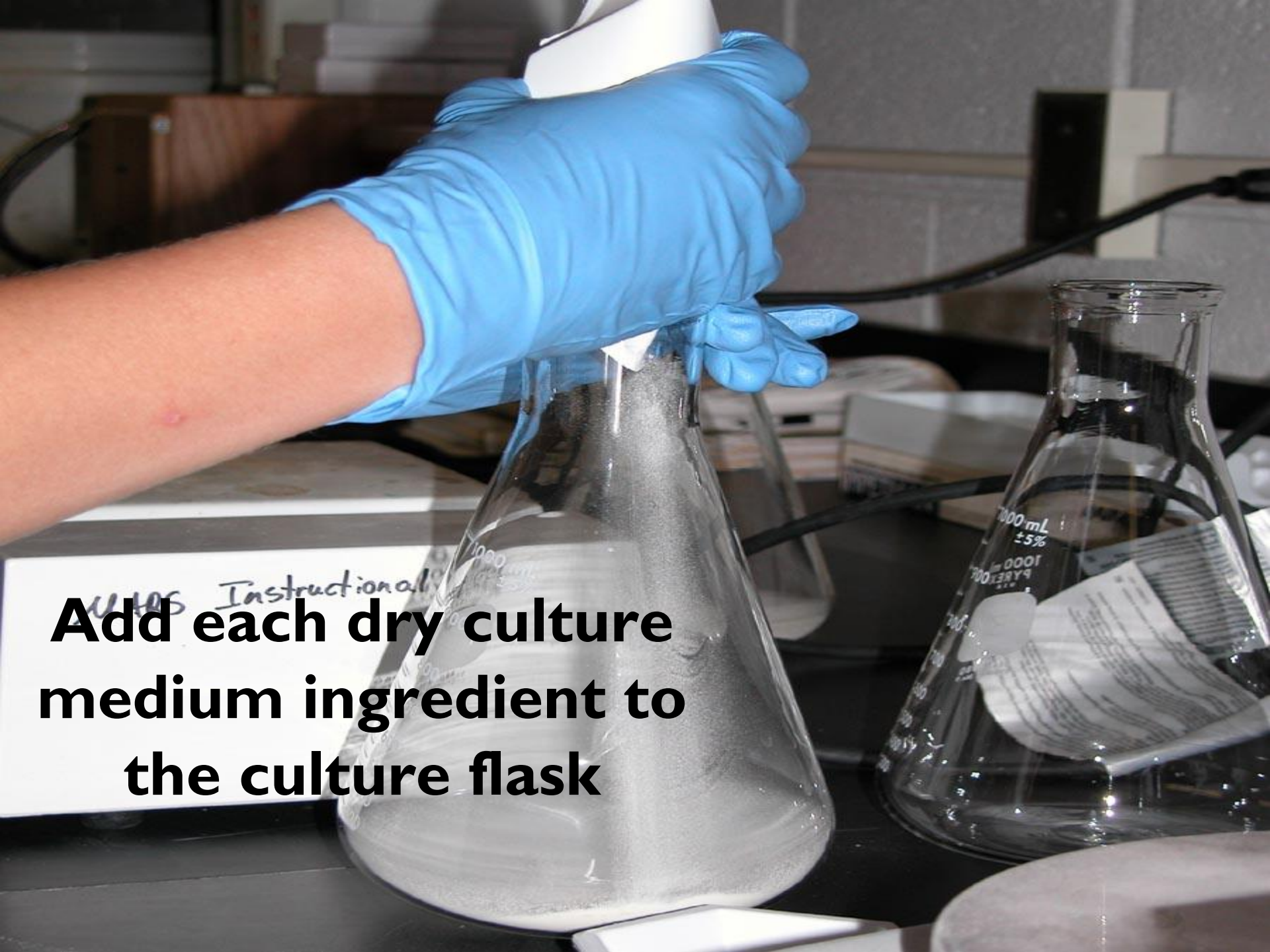
Media Preparation

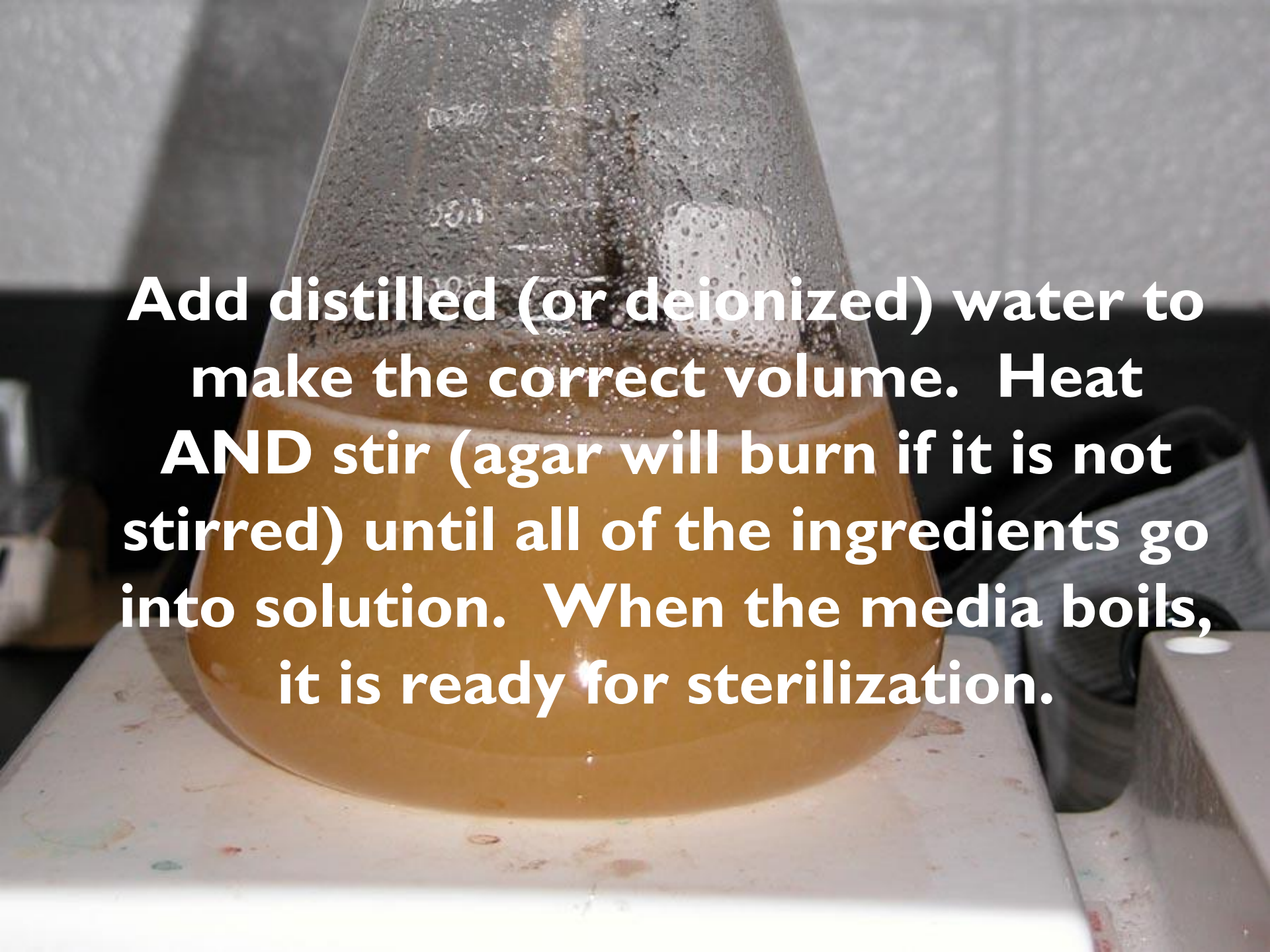
Microbiological Media

- The type of growth medium that you use is a function of the organisms that you want to culture. Use a reference book (there are many) to determine the type of medium that is best suited for your organism of interest.
- Common media include *Czapek's Dox Agar Medium, Sabouraud's Dextrose Agar, Potato Dextrose Agar* (PDA), *Martins Medium* etc.

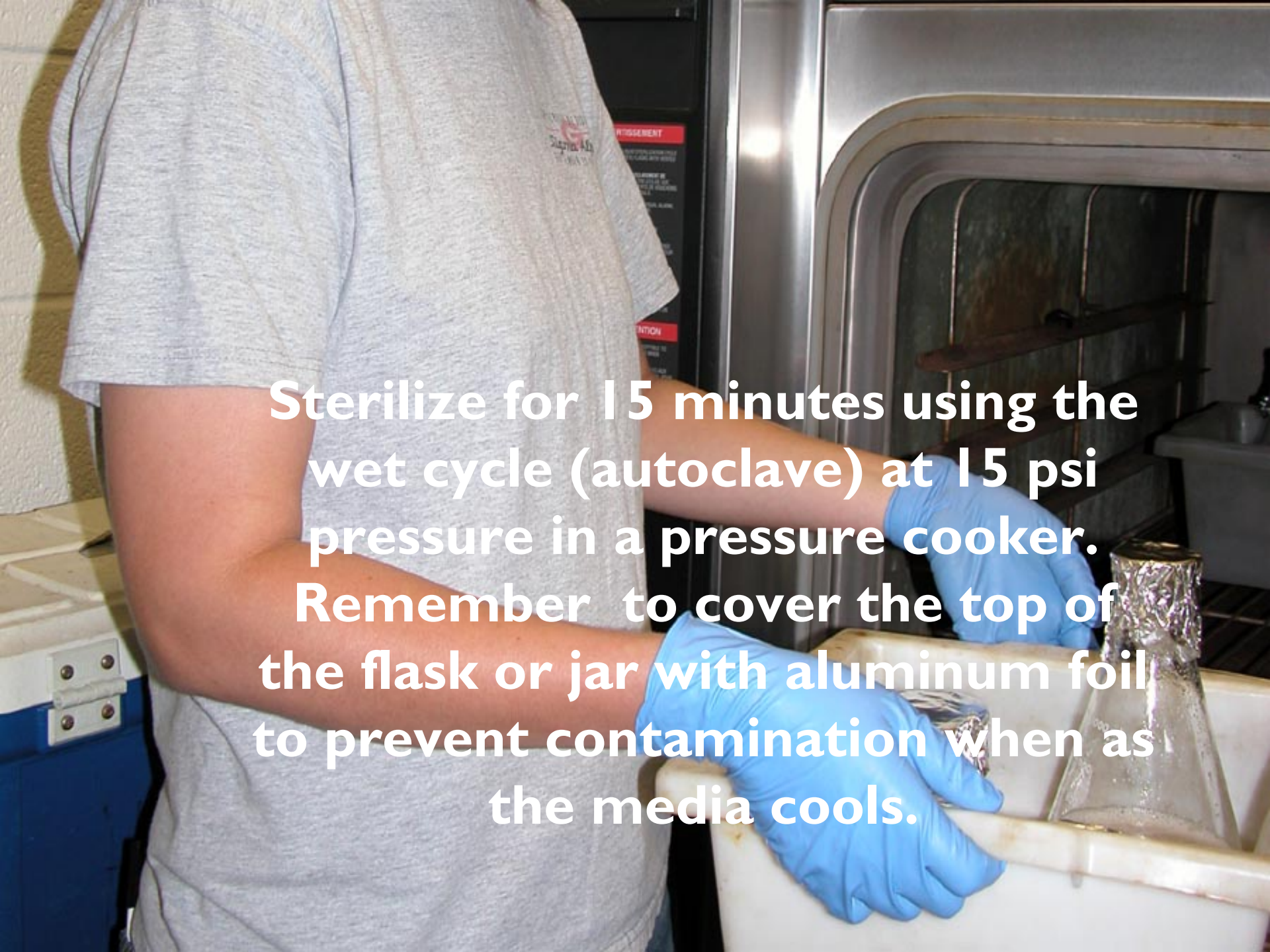
Assemble all of your chemicals in your work area before you begin.


Accurately weigh each of the dry ingredients in your culture media.


Add each dry culture medium ingredient to the culture flask

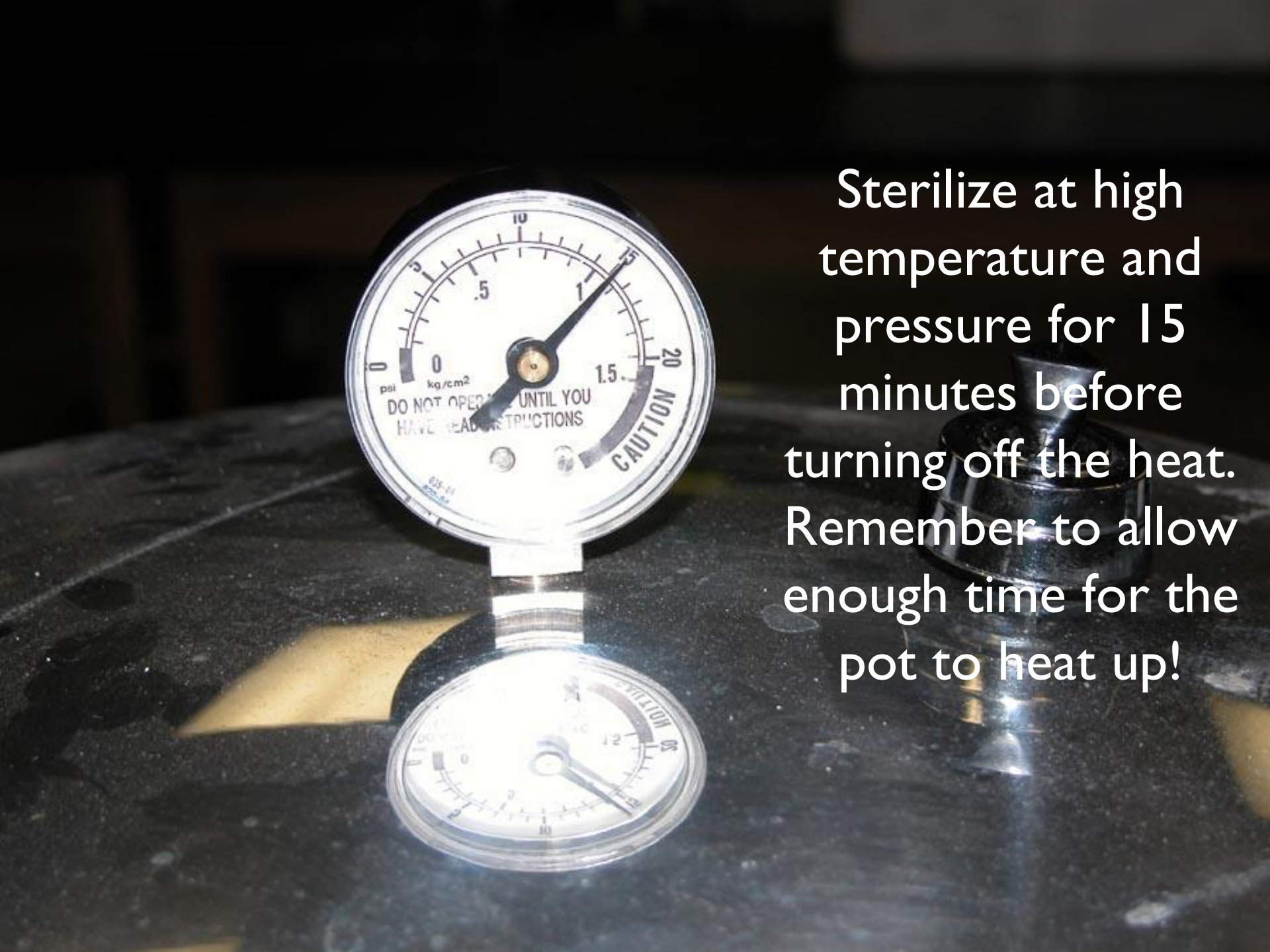


Add distilled (or deionized) water to make the correct volume. Heat AND stir (agar will burn if it is not stirred) until all of the ingredients go into solution. When the media boils, it is ready for sterilization.



Sterilize for 15 minutes using the wet cycle (autoclave) at 15 psi pressure in a pressure cooker. Remember to cover the top of the flask or jar with aluminum foil to prevent contamination when as the media cools.

When using a pressure cooker, don't over fill the cooker, and remember to weight your containers so they don't fall over!



Sterilize at high temperature and pressure for 15 minutes before turning off the heat. Remember to allow enough time for the pot to heat up!

Sterilization Techniques

- When culturing fungi or other microorganisms, it is important to keep your work area as clean as possible.
- This prevents the introduction of other microorganisms from the environment into your culture.
- The techniques used to prevent contamination are referred to as **sterilization techniques.**

1. Start by washing your down your work or lab benches with a surface disinfectant. The most commonly used disinfectants for lab use are:
   i. 10% bleach (recommended by the CDC)
   ii. 85% ethanol

2. Turn off any forced air heating or air conditioning units that create strong air current in your work area.
3. A small room or closet that can be closed off is worth the effort to set-up if you will be doing a lot of microbial culturing.
4. You can install a UV bulb in a fluorescent light fixture to surface sterilize your work bench if you have an enclosed area. Remember to leave the area when you turn on the UV light source!

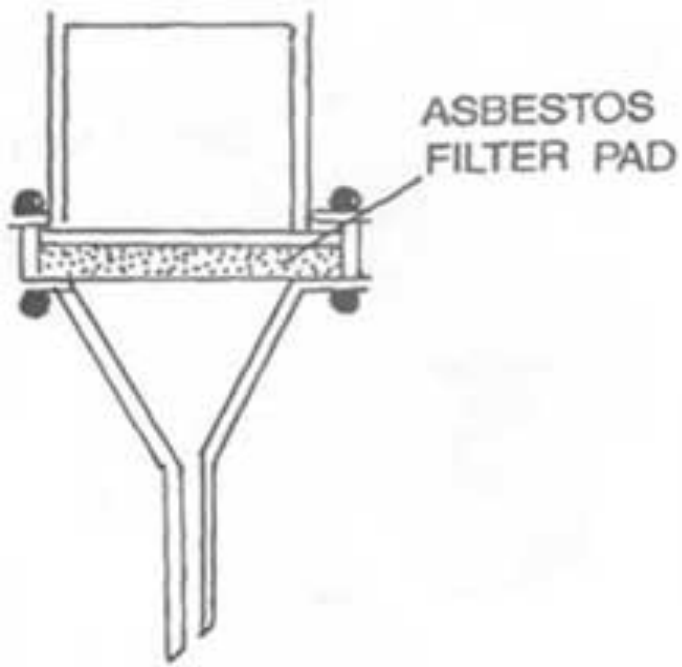
5. All glassware should be cleaned and sterilized before you begin.
6. All pipettes, spatulas, and test tube (culture) racks should also be sterilized.
7. You can purchase sterile, disposable culture tubes, petri dishes, and pipettes to minimize the quantity of glassware that you have to sterilize.

8. Don’t forget to wash you hands after you finish cleaning and put on a pair of sterile disposable gloves before you begin.
9. Once your work area is clean, your hands are clean, and your glassware is clean and sterile, don’t contaminate the work area by placing “dirty items” such as pencils, pens, notes, or books in the sterile work area.

Autoclave

Hot Air Oven

Seitz Filter



Membrane Filter

Laminar Air Flow

Plate Pouring Tips

- Line empty plates along the edge of the work bench.
- Open the petri dish lid at about a 30-45° angle to allow the hot liquid to cover the bottom of the dish. The thermal current created by the hot media prevents bacteria and fungal spores from landing in your clean dish.

Line your sterile petri plates along the edge of the table. Transfer hot media to a small sterile container and pour 15-20 ml of the plate media into each petri plate. The petri plate lid should be open slightly, but not completely open as this increases contamination.

- As the plates are poured, move the filled plates to the back of the table until the plates cool and congeal.
- Once the plates have cooled and the media is firm, store the plates media side-up (bottom) with the lid securely taped or the plates restacked in the manufacturer's plastic sleeve.
- To increase the shelf-life of the plates, store in a cool, dry environment until they are used (refrigerator).

Inoculating Plates and Culture Tubes

Inoculation of Culture Plates and Tubes

- ✔ Clean and surface sterilize your work area as detailed in the section on *Sterile Technique*.
- ✔ Use either disposable inoculation loops or a metal loop that can be heat sterilized to inoculate plates, slants, and liquid culture tubes.
- ✔ If using a metal loop, be sure to cool the loop by touching the sterile cooled liquid media or the sterile culture plate **before** the placing the loop in your live culture. Failure to cool the loop will kill your active microbial cultures!

Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 1: Remove the culture tube stopper or cap with one (do not set it down) and flame the mouth of the tube to surface sterilize the mouth. The heated tube surface will generate a thermal current that prevents contamination of the culture.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Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 2: Without setting any of the culture materials on the bench, place the sterile inoculation loop in the culture.

Step 3: Replace cap on the culture tube with the active microbes and put it in the test tube rack.

Step 4: Without setting the loop down, pick-up a sterile fresh culture tube with media with one hand, and remove the cap with the other hand.

Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 5: Flame the mouth of the clean culture tube.

Step 6: Place the inoculation loop containing the microbes in the fresh media and swirl the loop in the loop in the media to ensure even dispersal in the media.

Step 7: If using a solid media slant tube, follow steps 1-5 and then zig-zag the inoculation loop across the slanted surface of the solid media in the tube.


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Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 8: Flame the mouth of the newly inoculated culture tube and replace the cap.

Step 9: Place the culture tube in test tube rack.

Step 10: Repeat until all of the sterile tubes have been inoculated. Use a fresh disposable culture loop for each tube or flame the metal loop after each tube has been inoculated.

Inoculation of Liquid and Solid (Slant) Culture Tubes

Step 11: Incubate the culture at the recommended temperature (check with your supplier for growth requirements). If using environmental samples, incubation at room temperature will avoid the accidental culture of human pathogens.

Step 12: Dispose of all culture materials in a biohazard bag and sterilize all old cultures before pouring out cultures and washing culture tubes. Disposable culture dishes should be melted in an autoclave or pressure cooker prior to disposal.


Autoclave Bag
Fish

Inoculating Petri Plates

Step 1:Remove the culture tube stopper or cap with one (do not set it down) and flame the mouth of the tube to surface sterilize the mouth. The heated tube surface will generate a thermal current that prevents contamination of the culture.

Step 2: Without setting any of the culture materials on the bench, place the sterile inoculation loop in the culture.

Step 3: Replace cap on the culture tube with the active microbes and put it in the test tube rack.

Inoculating Petri Plates

Step 4: Holding the petri dish lid at an 30-45° angle, work the inoculating loop from the outside of the plate toward the center in a zig-zag pattern that covers approximately 25% of the plate surface (think pie or pizza slice!).

Inoculating Petri Plates

Step 5: Turn the petri plate 90° to the right, dragging the inoculation loop through the last section of the plate, moving from the outside to the inside in a zig-zag motion.

Step 6: Repeat this process twice more until the entire plate surface is covered.

NOTE: If you are trying to isolate individual colonies, each turn of the dish will give you fewer microbes so that you can distinguish individual colonies.

B.O.D. Incubator

Colony Counter

Scope of Microbiology

- At least a bachelor's degree in microbiology or a closely related field is required for would-be microbiology scientists.
- A concrete foundation of the sciences is needed. Microbiology majors pursue courses such as virology, microbial chemistry, biochemistry, physiology, and physics.
- A number of these courses comprise a laboratory component, which is crucial for soon-to-be microbiologists.
- Microbial genetics and microbial physiology are the core courses taken by most microbiology majors, while elective classes are such as environmental microbiology and virology.

- Employment of microbiologists is estimated to grow nearly 8 percent over the coming ten years, which is as fast as the average for all professions.
- More microbiologists will be required for contribution to basic research and for solving glitches of industrial production. There will be a need for microbiologists to conduct research and thus develop novel medicines and treatments, such as antibiotics and vaccines.
- Besides, pharmaceutical and biotechnology companies will need microbiologists to develop drugs which are produced with the service of microorganisms.

Employment Areas for Microbiologists:

Microbiologists mainly worked in laboratory research settings in decades past. With the potential role of microbes in biotechnology as well as their new appreciation in environmental and human health, microbiologists today work as intact members of interdisciplinary squads in clinics, hospitals, industry, universities, and government. They are on the cutting edge of science.

The significant employers of microbiologists are as follows:

The state government, excluding education and hospitals—18%

Universities, colleges, and professional schools; state, local and private—14%

Medicine manufacturing and pharmaceutical—32%

Research and development in life sciences—36%

Thank you