* Waste Disposal:
  + Most materials are sterilized prior to use and before final disposal
  + Common sterilization methods:
    - Autoclave
    - Incineration
    - UV cross-linking
  + Disposal rules:
    - Biohazard: anything in contact with cultures, Petri plates, swabs, and pipette tips
    - Glass box: broken glass, glass pipette tips
    - Regular trash: paper towels, gloves that have only touched stains, lens paper, bibulous paper
* “Important Terminology”:
  + Microbiology: the study of organisms that can only be viewed under the microscope
    - Medical microbiology: study of disease-causing microbes
      1. Nosocomial: an infection not present upon admission to a hospital but incurred while being treated there
      2. Aseptic: free of pathogenic organisms
    - Environmental microbiology: study of microorganisms outside of medical importance
  + Microorganisms: single-celled, clustered, or multicellular organisms
  + Containment:
    - Primary: protection of lab personnel and lab equipment
    - Secondary: protection of outside lab environment from exposure to infections organisms
  + Germ theory: microorganisms are the causative agents for many diseases and the spread of disease goes hand in hand with their transmission
    - Koch’s Postulates:
      1. Association of microorganism with disease (present in all cases)
      2. Isolation of microorganism from diseased host(s)
      3. Inoculation (implantation of microorganisms into or upon culture media) of isolated microorganism to see if it causes disease
      4. Re-isolation of microorganism from diseased host(s)
  + Turbid: cloudy appearance of a nutrient solution in a test tube due to growth of microbe population
  + Microbial Colonies:
    - Colony: a visible cluster of microbes growing on solid media
      1. Single cell reproduces asexually until it is visible
      2. Rough biomass estimate can be gotten via counting colonies
* Staining:
  + Varieties:
    - Simple: staining to detect the *presence* of microorganisms
    - Differential: staining designed to distinguish types of microorganisms based on some identifying feature (ex. Gram staining)
    - Negative: staining of everything *around* the microorganism
    - Positive: staining that penetrates the membrane(s) of the microorganism(s) to stain the organism(s) themselves
* Pure Culture Techniques:
  + Streak plate method: pure culture technique used to separate mixed cultures or assess purity
    - Sufficiently isolated cells will grow into colonies consisting of genetic copies of the original cell known as colony forming units (CFUs)
  + Spread plate: deposition and uniform spread of a diluted microbial sample across an agar plate with an ethanol-sterilized glass rod
* Coliforms: group of bacteria widely distributed in the environment and in the digestive system of birds and mammals
  + Indicator organisms: microorganisms that are markers for possible fecal contamination when found in water
    - Good indicators b/c they are easily grown and identified in the lab
  + MPN technique: quantitative technique utilizing serial dilutions and presence/absence data to estimate the most likely number of CFUs per 100mL of water
    - Durham tubes: small tubes used to indicate the presence or absence of gas accumulation
    - LTB: lauryl tryptose broth containing lauryl sulfate and lactose
      1. Lauryl sulfate inhibits the growth of non-coliforms
      2. Lactose is used as fermentation source for coliforms
    - BGB: brilliant green bile broth
  + Membrane filtration technique: vacuum filtration of water sample and subsequent inoculation of bacteria on an Endo agar plate
    - Endo agar: nutrient agar selecting for Gram negative organisms and containing lactose and fuchsine (stain that labels as pink/red organisms that produce lactic acid)
      1. Non-coliform colonies will appear white/clear
      2. Coliform colonies will appear as pink/red with a green metallic sheen
* Oxygen Tolerance:
  + Organism types:
    - Obligate aerobe: organisms that require oxygen as a final electron acceptor
    - Obligate anaerobe: organisms that are incapable of using oxygen as a final electron acceptor and poisoned by it
    - Facultative aerobe: organisms that use oxygen as a final electron acceptor in aerobic environments
    - Aerotolerant: organisms that never use oxygen as a final electron acceptor but can detoxify toxic forms of oxygen
    - Microaerophile: organisms that require oxygen as a final electron acceptor at low concentrations because they have only a limited ability to detoxify toxic forms of oxygen
  + Testing:
    - Done using FTM (fluid thioglycolate media), which provides an oxygen gradient and indicator
      1. *S. epidermidis* was the obligate aerobe in this lab
      2. *C. sporogenes* was the obligate anaerobe in this lab
* Gram Staining: differential stain that colors bacterial cells based upon the morphology of the cell wall
  + Component stains:
    - Primary: crystal violet
    - Mordant: Gram’s iodine
    - Decolorizer: alcohol
      1. This step is where Gram positive and Gram negative cells are differentiated (Gram+ stay blue while Gram- decolorize)
      2. Over-decolorization results in false negatives
    - Counterstain: Safranin
      1. Gram+ cells stay blue while Gram- cells turn pink/red
* MPN: most probable number
  + Formula: MPN/100mL = 100P + rad(Vn Va)
    - P = total number of positive results
    - Vn = combined volume of sample that produced negative results
    - Va = combined volume of sample in all tubes
* Enterobacteriaceae: large family of Gram-negative bacteria that are a normal part of gut flora and can also cause gastrointestinal illnesses
  + Testing is often based on changes in pH due to production of acidic or alkaline byproducts
    - Color change usually indicates a positive result
    - Differential media: media that allows the growth of several types of organisms but also allows distinguishing of said organisms based on different phenotypic traits that are displayed
      1. MacConkey Agar is an example of differential media
         * Bile salts select for gram-negative organisms
         * Lactose causes red color change in organisms that ferment lactose (organisms that are nonlactose fermenting stay colorless)
      2. Kliger’s Iron Agar
         * Differentiates between organisms that ferment lactose (and glucose) and those that *only* ferment glucose, and those who cannot ferment *either* sugar

Media remains red if neither sugar is fermented

Slant remains red while butt of media turns yellow if only glucose is fermented

Slant and butt turn yellow if lactose is fermented due to lactic acid formation

Media turns black if a sulfur-reducing organism is present

* + - 1. Sulfur Indole Media
         * Designed to test for indole production, motility, and H2S production

Addition of Kovac’s Reagent tests for indole production (surface of agar will turn red if it is present)

Organisms that are motile will exhibit diffuse growth spreading out from the initial stab line; organisms that are not motile will only grow where the agar was stabbed

Presence of H2S gas will give a black precipitate (reaction with ferrous sulfate occurs)

* + - 1. Urea Broth:
         * Tests for the hydrolysis of urea to carbon dioxide and ammonia (rapid pH change occurs)

Test turns pink in the presence of urease

Solution remains yellow/orange if urease is not present

* + - 1. Citrate Utilization:
         * Tests the ability of the organism to use citrate as the sole carbon and energy source

Positive reaction turns bromothymol blue indicator from green to blue

Negative result remains green

* + - 1. Methyl Red Voges-Proskauer:
         * Tests whether a species ferments glucose to produce stable organic acids or whether it produces a neutral alcohol end product

Methyl red indicator turns red when acids are produced and has no color with a neutral alcohol end product

Voges-Proskauer used on negative methyl red tests; cherry red color produced in presence of acetoin while a negative reaction turns copper

* + - 1. Oxidase Test:
         * Tests for the presence of cytochrome C oxidase in the electron transport chain

Oxidase positive organisms will turn the swab from orange to purple

* + - 1. API strips can also be used for the identification of *Enterobacteriaceae* (each microtubule has a specific dehydrated media and color changes take place in the tubes during incubation or after reagent addition)
         * Unique code from tests entered into API database to come up with the nearest organism and confidence
* Transformation: when a cell takes up and expresses a new piece of genetic material
  + Often provides organism w/a new trait identifiable after transformation
  + Plasmid: DNA molecule that is separate from and can replicate independently of chromosomal DNA
    - Provide a natural mechanism for horizontal gene transfer and typically provide a selective advantage
    - May carry:
      1. Antibiotic resistance genes
      2. Toxin production genes
      3. Ability to fix elemental N
      4. Ability to degrade recalcitrant organic compounds when nutrients are scarce
    - There are both integrating (within the host chromosome) and non-integrating plasmids
  + Green Fluorescent Protein: (GFP) a protein produced by the bioluminescent jellyfish that is integrated into many plasmids by biotech companies for confirm a bacterium was successfully transformed
    - pGLO plasmid contains the araC gene (arabinose sugar usage), a GFP gene, and the beta-lactamase gene for ampicillin (is an antibiotic) resistance
  + “Terms to Know”:
    - Media: liquid and solid nutrient media referred to as LB agar
    - Transformation solution: solution with Ca2+ cation that neutralizes the repulsive negative charges of the phosphate backbone of DNA and the phospholipids of the cell membrane to allow the plasmid DNA to enter the cells
    - Heat shock: temperature spike to increase the permeability of the cell membrane to DNA
    - Recovery: ten-minute incubation period following addition of LB broth allowing cells to recover and express ampicillin resistance gene so that transformed cells survive on ampicillin selection plate
* Germicides:
  + Best disinfectants and germicides are:
    - Broad spectrum
    - Stable
    - Non-staining
    - Non-corrosive
    - Relatively safe for users
  + Disinfectant: growth-inhibiting agent used on *fomites* (inanimate objects) (ex. ethanol and bleach)
    - Sometimes lower concentrations of disinfectant are better for killing bacteria (also cheaper)
  + Antiseptic: growth-inhibiting agent used on living tissues to prevent infection (ex. listerine)