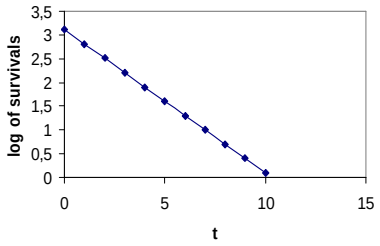
PRACTICE 4

Sterilization and Disinfection

Antimicrobial kinetics



$S = S_0 \times e^{-kt}$

-k: death rate

S_0: germ number at start

S: germ number at time t

STERILIZATION

- Sterilization is the **killing** of all microorganisms in a material or on the surface of an object
- Typically the last things to die when one attempts sterilization is the highly heat- (and chemical-, etc.) resistant endospores

DISINFECTION

- Disinfection means **reducing the number** of viable microorganisms present in a sample
- A **disinfectant** is a chemical or physical agent that is applied to inanimate objects to kill microbes
- Not all disinfectants are capable of sterilizing, but, of course, all disinfectants are employed with the hope of disinfecting
- Spores and some bacteria, viruses and fungi may survive

DISINFECTION

- Typically an **antiseptic** is a chemical disinfectant agent that is applied on skin or mucous membranes to kill microbes
- An antiseptic should not be so harsh that it damages living tissue !

BACTERICIDAL EFFECT:

An antimicrobial that kills a microorganism (or, more specifically, a bacterium) is said to be bactericidal

BACTERIOSTATIC EFFECT

Bacteriostatic means that the antimicrobial inhibits bacterial growth but does not kill the bacteria; consequently, removal or dilution of the antimicrobial can result in a resurgence of bacterial growth

Some antimicrobials are only bacteriostatic, including some bactericidal agents used at too-low concentrations

HEAT STERILIZATION AND DECONTAMINATION

- Red-heat flame
- Dry heat (Hot Air Oven, Dry Heat Sterilizator)
- Moist heat (pasteurization, tyndallization, boiling, autoclaving)

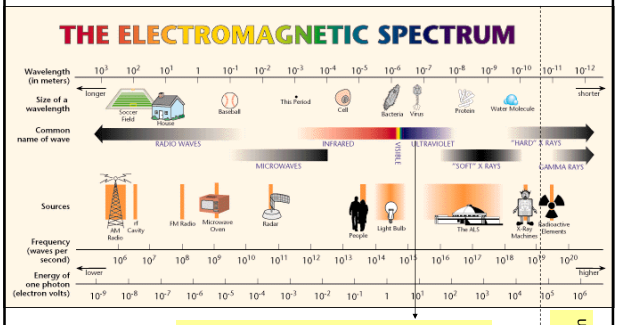
HOT AIR OVEN, DRY HEAT STERILIZATOR

160°C for at least 60 min
180°C for at least 20 min

MOIST HEAT: AUTOCLAVE

Temperature °C	Pressure kPa	Overpressure kPa	Exposition time
120	200	100	20 min
125	240	140	15 min
134	300	200	10 min

Using electromagnetic irradiation



At 254 nm wavelength it damages DNA but it can exert its effect only on surfaces & in air

not for sterilization, rather for sanitation

reliable sterilization

OTHER PHYSICAL METHODS

- ULTRAVIOLET LIGHT (germicidal lamps)
- GAMMA IRRADIATION
- PLASMA STERILIZATION
- MEMBRANE FILTRATION

MONITORING THE EFFECTIVENESS OF HEAT, RADIATION STERILIZATION

Bacillus stearothermophilus, B. subtilis spores:
= biological indicators

CHEMICAL STERILANTS AND DISINFECTANTS

- Disruption of cell membranes
  (alcohol, detergents, phenols)
- Modification of proteins
  (chlorine, iodine, heavy metals, H_2O_2, aldehydes, ethylene oxide, beta-propiolactone, acids, and alkalis)
- Modification of nucleic acids
  (some dyes: christal violet, malachite green)

SOAPS
= sodium or potassium salts of fatty acids

- Anionic detergents
- Consequently, soaps are alkaline (pH greater than 7)
- Soaps exert their antimicrobial effects in two ways
  - By harming bacteria that are sensitive to high pH's
  - By removing pathogens from surfaces by cleaning the surface (a.k.a., degerming)
- BACTERIOSTATIC effects

SKIN ANTISEPTICS can contain eg.

- Cationic detergents (quaternary ammonium compounds)
- alcohols

Phenol coefficient

• A measure of the bactericidal activity of a chemical compound in **relation to phenol**
• The coefficient is calculated by dividing the concentration of the **test compound** at which it kills the test organism in 10 minutes, but not in 5 minutes, by the concentration of **phenol** that kills the organism under the same conditions.
• The test is performed under **standardized conditions** using different test bacteria:

- *Staphylococcus aureus*
- *Salmonella typhi*
- *Pseudomonas aeruginosa*

EFFECTIVENESS of DISINFECTANTS on BACTERIAL FLORA of SKIN

• Label the plate with blood agar with your name and divide the plate into 4 quarters

- I: finger impression
- II: finger impression after hand-washing with soap
- III: finger impression after disinfection 1X
- IV: finger impression after disinfection 3X