PRESERVATION OF PHARMACEUTICAL PRODUCTS

INTRODUCTION

- **Preservatives are the chemical substances used to improve or amplify shelf life of drugs by decreasing or lowering the oxidation of active ingredients or excipients and by reducing microbial production**
- **Prevent the growth of unwanted microorganisms in pharmaceutical products**

Ideal properties of preservatives:

- ❖ **Should able to kill all microbes contaminants rapidly**
- ❖ **Tasteless and odourless**
- ❖ **Not be irritant**
- ❖ **Non toxic to the patient**
- ❖ **Physically and chemically Stable**
- ❖ **Effective at low concentration throughout the life of the medicine**
- ❖ **Should be selective in reacting with the contaminants and not the ingredients of the medicine.**
- ❖ **Cost effective**

❖ A single preservative is not suitable for preservation of all pharmaceutical formulations.

- Some preservatives are ineffective with some microbe strains and should be combined with others to be effective.
- Combination of two or more preservatives are used to extend the range and spectrum of preservation.(Synergic action)

✓ **Germall 115+parabens (antibacterial +antifungal)-Tablets,**

✓ **Phenylethyl alcohol+phenoxetol+ benzalkonium chloride (wide antimicrobial) -Eye drops and contact lens**

✓ **Methyl parahydroxybenzoic acid and propyl parahydroxybenzoic acid (antimicrobial) –Injection**

- It must decrease the percentage of the microbes and prevent any re-growth, They can be:
- **–Microbiostatic- that inhibits the growth or multiplication of microbiota(pathogenic microbes)**
- **–Microbiocidal in nature- that kills the microbiota**

NEED FOR PRESERVATIVES

- To protect our drug from microbial attack
- To enhance activity and efficacy of drug
- To increase shelf life of our product
- To stabilize our product

Classification of preservation

- **Based on mechanism of action:**

1. **Antioxidants:** The agent which prevent oxidantion of API which otherwise undergo degradation due to oxidation as they are sensitive to oxygen.

Ex: Vitamin E & C, Butylated hydroxy anisole (BHA), Butylated hydroxy toluence(BHT).

2. **Antimicrobial agents:** the agent which active against Gram +ve and Gram –ve microbes which causes degradatation of pharmaceutical preparation which are active in low concentration.

Ex: benzoates, sodium benzoate, sorbates.

3. **Chelating agents:** the agents which form the complex with pharmaceutical ingredient and prevent the degradation of pharmaceutical formulation.

Ex: EDTA, Polyphosphates, citric acid

Classification of preservation

➢ **Based on sources:**

1. **Natural preservatives: These preservatives are obtained from natural sources like plants, minerals and animal sources, etc**

Ex: Neem oil, salt(sodium chloride), lemon, honey

2. **Artificial preservatives: These are man made by chemical synthesis and active against various microbes in small concentration.**

Ex: Benzoates, sodium benzoate, sorbates, propionets, nitrites.

Chemical preservatives used in pharmaceutical formulation

Table 15.1: Preservatives used in pharmaceutical formulations

Formulation	Preservative	Concentration (% w/v)
Tablets	Methyl paraben	0.1
Injections	Phenol	0.2 – 0.6
	Cresol	0.2 – 0.5
	Benzyl alcohol	1.0 – 2.0
	Thiomersal	0.01
	Methyl Hydroxybenzoate	0.1
Eye drops	Benzalkonium chloride	0.01
	Phenylmercuric nitrate	0.002
	Chlorhexidine acetate	0.01
Liquids/mixtures	Bronopol	0.02
	Alcohol	15 – 20
	Methyl paraben	0.1
	Chloroform	0.25
	Benzalkonium chloride	0.005 – 0.02
	Chlorocresol	0.1
Semisolids	Chlorocresol	0.2
	Dichlorobenzyl alcohol	0.1 – 0.2
	Cetyltrimethyl ammonium bromide	0.05 – 0.1

PERFORMANCE REQUIREMENTS

Antimicrobial Activity	• Active Against Microbes at Low Concentration
Aqueous Solubility	• Should Be Soluble To Reach Minimum Inhibitory Concentration
Stability Properties	• Stable During and at The End of Manufacturing
Partitioning behaviour	• Remain in continuous phase in multiphase products
Organoleptic properties	• Odour and acceptable taste during administration of the product

FACTORS AFFECTING PRESERVATIVES EFFICACY

1. Interaction with formulation

2. Properties of the preservation
3. Effects of containers
4. Type of microorganisms
5. Influence of pH

1. Interaction with formulation

- Hydrocollids such as **methylcellulose, alignates, tragacanth can interacts with** preservatives and **diminish their activity.**
- Many emulgents are used in pharmaceutical preparations to produce elegant applications. Interaction may occur between preservatives and emulsified oil phase and with emulgent molecules.
- Nature of oil, oil water ratio, type of concentration of emulgent, influence the concentration of preservatives needed to protect the system.
- Many **tablet additives cause problems in tablet preservations due to their interaction with added preservatives.**

2. Properties of the preservation

- **The distribution of preservative must be homogeneous and more solubility in the bulk phase is preferable in multi phase system.**
- Some chemicals such as **chlorobutol may hydrolyse on storage if the** pH is unfavourable.
- Preservatives may **react with substances leached from the container** and lose its antimicrobial activity.

3. Effects of containers

- Formulations packed in **glass containers can be expected to retain their preservative content if closure is airtight.**
- Preservatives may **penetrate through the plastic container and interacts with it.**
- **Rubber also reacts with many preservatives but is still used for** closures.
- Containers or closures may cause contamination of pathogens.
- Screw–capped containers and corks are the common source of mould spores.

4. Type of microorganisms

- **Plants products may contain pathogenic microorganisms from the soil. E.g Clostridium species, Bacillus anthracis.**
- **These soil** microorganisms can cause spoilage of pharmaceutical products.
- Many **products prepared from animal sources may contain pathogens like Salmonella typhi.**
- Spores of tetanus and gas gangrene have been isolated from geletin.

5. Influence of pH

- Adjustment of the pH of solution may affect the chemical stability and the activity of the preservative.
- The **majority of preservatives are less dependent upon pH, although** cationic active quaternary ammonium compounds are more active at high pH values.

ANALYSIS OF PRESERVATIVES IN PRODUCTS

High performance liquid chromatography
Capillary electrophoresis (CE)
Gas chromatographic methods
Thin layer chromatographic methods
Flow injection analysis

ANALYSIS OF PRESERVATIVES IN PRODUCTS

Titrimetric Methods
Flourimetric Methods
Spectrophotometric methods
Atomic Absorption Spectroscopic (AAS) Method

SIDE EFFECTS

- While choosing preservative for drug product consideration should be made about:
- **1. Concentration**
- **2. Toxicity**
- **3. Selectivity**
- **4. Interaction with formulation, etc.**

Some preservatives and their Side effects

Sl. no	Preservative	Side effects
1.	Paraben	Neurological damage in rats Potent irritants
2.	Formaldehyde Diazolidinyl urea Imidazolidinyl urea	Skin irritants Eye irritants lung irritants
3.	Benzyl alcohol	fatal toxic syndrome in low weight neonates
4.	Cetyl alcohol Stearyl alcohol	Infrequent sensitizers
5.	2-Phenylethanol	Irritant to skin, eye and mucous membranes
6.	Benzoic acid	Gastro-irritant
7.	Chloroxylenol	Cross sensitivity
8.	Chlorocresol	Irritant to skin, eyes and mucous membranes
9.	Hexachlorophene EDTA	Neurotoxicity Dose-related bronchoconstriction

EVALUATION OF PRESERVATIVES

- The evaluation of preservatives has traditionally involved time-consuming tests:

1. Preservative Efficacy Tests(PET) also called Antimicrobial Effectiveness Testing(AET)

➢ A number of factors determine the efficacy of a preservative, including the active component of the product, the formulation in which it is incorporated or the container/packaging in which the product is enclosed.

Why needed?

❖ Antimicrobial preservatives are added to **pharmaceuticals, medical devices, personal care products, cosmetics and food products to inhibit the growth of micro-organisms inadvertently introduced during the manufacturing process or during the use of products with multiple-dose containers.**

❖ **Guaranteeing that no inappropriate organisms are able to sustain themselves within the product or its packaging.**

❖ Is undertaken to ensure that the **preservative maintains its ability to inhibit micro-organisms and is required by British Pharmacopoeia (BP), European Pharmacopoeia (Ph. Eur.) and United States Pharmacopoeia (USP) standards.**

Preservative Efficacy Test (PET)

- First appeared in USP in 18th revision, 1st sept. 1970.
- Applied to the **formulated medicine in its final container to determine whether it is adequately protected from microbial spoilage.**
- **Tests/standards apply only to the product in original, unopened containers**
- This test is done to determine the **efficacy of preservative against microorganism.**
- Involve challenging a product with a defined number of colony forming units (CFU) of a variety of test microorganisms (bacteria, yeasts and fungi)
- Then monitoring the kill /survival rate at defined time intervals up to 28- days.
- It is examined by the duplicate plate count method **(CFU/ml = number of colonies per ml plated /Total dilution factor)**
- Multiple dosages forms- Parenterals, otics, nasal, oral, topical and ophthalmic products made with aqueous bases or vehicles.

STEPS INVOLVED

- **Preservative efficacy test is done in following steps-**

1. **Product category**
2. **Test organism**
3. **Media culture preparation**
4. **Preparation of inoculum**
5. **Procedure**
6. **Interpretation**

1. Product categories

Table 1. Compendial Product Categories

Category	Product Description
1	Injections, other parenterals including emulsions, otic products, sterile nasal products, and ophthalmic products made with aqueous bases or vehicles.
2	Topically used products made with aqueous bases or vehicles, nonsterile nasal products, and emulsions, including those applied to mucous membranes.
3	Oral products other than antacids, made with aqueous bases or vehicles.
4	Antacids made with an aqueous base.

2. Test organisms

- **Choice of test microorganisms: Preservatives should be active against as wide as a range of microorganisms as possible hence the choice should be of both Gram+ve and Gram-ve bacteria, yeast and moulds in IP Test.**
- Test organism that are recommended by all of the pharmacopoeias includes:

✓ ***Staphylococcus aureus ATCC 6538***

✓ ***Pseudomonas aeruginosa ATCC 9027***

✓ ***E. coli ATCC 8739***

✓ ***Fungi/mould, Aspergillus Brasiliensis ATCC 16404***

✓ ***Yeast, Candida albicans ATCC 10231***

3. Media culture

- **Soyabean casein digest agar medium (SCDM) & Sabouraud -Dextrose medium.**

Medium 2 (Soybean-Casein Digest Medium)

Composition	w/v
Pancreatic Digest of Casein	17.0 g
Pepsin Digest of Soybean Meal	3.0 g
Glucose monohydrate/anhydrous	2.5 g /2.3 g
Sodium chloride	5.0 g
Di potassium hydrogen phosphate	2.5 g
Purified Water	1000 mL
Polysorbate 80-	5.0 mL

pH after sterilization (measured at room temperature): 7.3±0.2

which will support the growth of both aerobic bacteria (incubation temperature 30–35°C) and fungi (incubation temperature 20–25°C)

- http://www.himedialabs.com/TD/MM011.pdf

4. Preparation of inoculum

- Preparatory to the test, inoculate the medium from a recently stock culture of each of the specified microorganisms(1ml).
- **Escherichia coli ATCC 4352, Pseudomonas aeruginosa ATCC 9027 and Staphylococcus aureus ATCC 6538** are inoculated in **Sabouraud-Dextrose medium** and incubated **at 32.5 ± 2.5°C for 3-5 days.**
- **Candida albicans ATCC 10231, Aspergillus brasiliensis ATCC 16404** are inoculated in **Soyabean-Casein digest medium** and incubated at **22.5 ± 2.5°C for 3-5 days for Candida albicans & 3-7 days for Aspergillus brasiliensis .**
- After this incubation period, microbial culture is harvested by **washing with sterile saline(0.9% w/v) to obtain a microbial count of about 10^8 CFU/ mL (**collect it in a sterile container and treat this as Stock solution of test solution)

Organism	Suitable Medium	Incubation Temperature	Inoculum Incubation Time	Microbial Recovery Incubation Time
Escherichia coli (ATCC No. 8739)	Soybean–Casein Digest Broth; Soybean–Casein Digest Agar	32.5 ± 2.5	18 to 24 hours	3 to 5 days
Pseudomonas aeruginosa (ATCC No. 9027)	Soybean–Casein Digest Broth; Soybean–Casein Digest Agar	32.5 ± 2.5	18 to 24 hours	3 to 5 days
Staphylococcus aureus (ATCC No. 6538)	Soybean–Casein Digest Broth; Soybean–Casein Digest Agar	32.5 ± 2.5	18 to 24 hours	3 to 5 days
Candida albicans (ATCC No. 10231)	Sabouraud Dextrose Agar; Sabouraud Dextrose Broth	22.5 ± 2.5	44 to 52 hours	3 to 5 days

5. Procedure

- Maintaine aseptic condition throughout the process and use hand glove rinsed with 70% Isopropyl alcohol
- Use **five Product containers** if the volume per container is sufficient.
- Reconstitute(restore) the product container if it is in dry powder form, as per the instruction on the label.
- Standardize the **volume of the inoculum** to be between **0.5% and 1.0%** of the volume of the product and the concentration to be between **1×10^5 and 1×10^6 CFU/mL of the product.**
- Add the **inoculum into the product containers using Sterile Syringe or Pipette.**
- Determine the initial count of the inoculated containers by Plate count Method.
- Incubate the inoculated containers at $22.5 \pm 2.5°C$

➢ .The container sampling intervals include:

1. **Category I products are sampled at 7, 14 & 28 days,**
2. **Category II-IV products are sampled at 14 & 28 days.**

✓ **Withdraw samples from containers at the intervals of 7, 14 and 28 Days depending on product categories and determine the number of CFUs of viable microbes per mL present at each of these sample containers by the Plate Count Method utilizing media with suitable in- activator (neutralizer)** in the diluents such as sterile normal saline (0.9 % w/v) with polysorbate-80 (0.05 % w/v) and 0.05 % Soya lecithin or with peptone (0.1 % w/v) and 0.05 % Soya lecithin or sterile normal saline (0.9 % w/v) with 0.05 % polysorbate-80.**(sometimes APIs or other excipients may show preservative effects to neutralised theses effects in-activator are added)**

✓ Express this in terms of **Log Reduction** and report as Complies if results are complying to the criteria as given in Table.

6. Criteria for antimicrobial effectiveness/INTERPRETATION

The criteria for microbial effectiveness are met if the specified criteria are met.

For Category 1 **BACTERIA**	3.0 log reduction in 14 days, no increase up to 28days
Yeast	No increase from initial count at 14 and 28days
For Category 2 BACTERIA	2.0 log reduction in 14days, no increase up to 28days
YEAST	No increase from initial count at 14 and up to 28 days
For Category 3 **BACTERIA**	1.0 log reduction in 14days, no increase up to 28days
YEAST/MOULDS	No increase from initial count at 14 and 28 days
For Category 4 **BACTERIA**	No increase from initial count at 14 and 28 days
YEAST	No increase from initial count at 14 and 28days

OR Interpretation(ANYONE)

1 **Bacteria: Not less than 1.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days.**

2 **Yeast and Moulds: No increase from the initial calculated count at 14 and 28 days.**

Log reduction

- The **log reduction** achieved by a decontamination process is a measure of how thoroughly the process reduces the concentration of a contaminant.
- It is defined as the common logarithm of the ratio of the levels of contamination before and after the process, so an increment of 1 corresponds to a reduction in concentration by a factor of 10.

$$\text{Log Reduction} = \log_{10}\left(\frac{A}{B}\right)$$

or,

$$\text{Log Reduction} = \log_{10}(A) - \log_{10}(B)$$

Where:

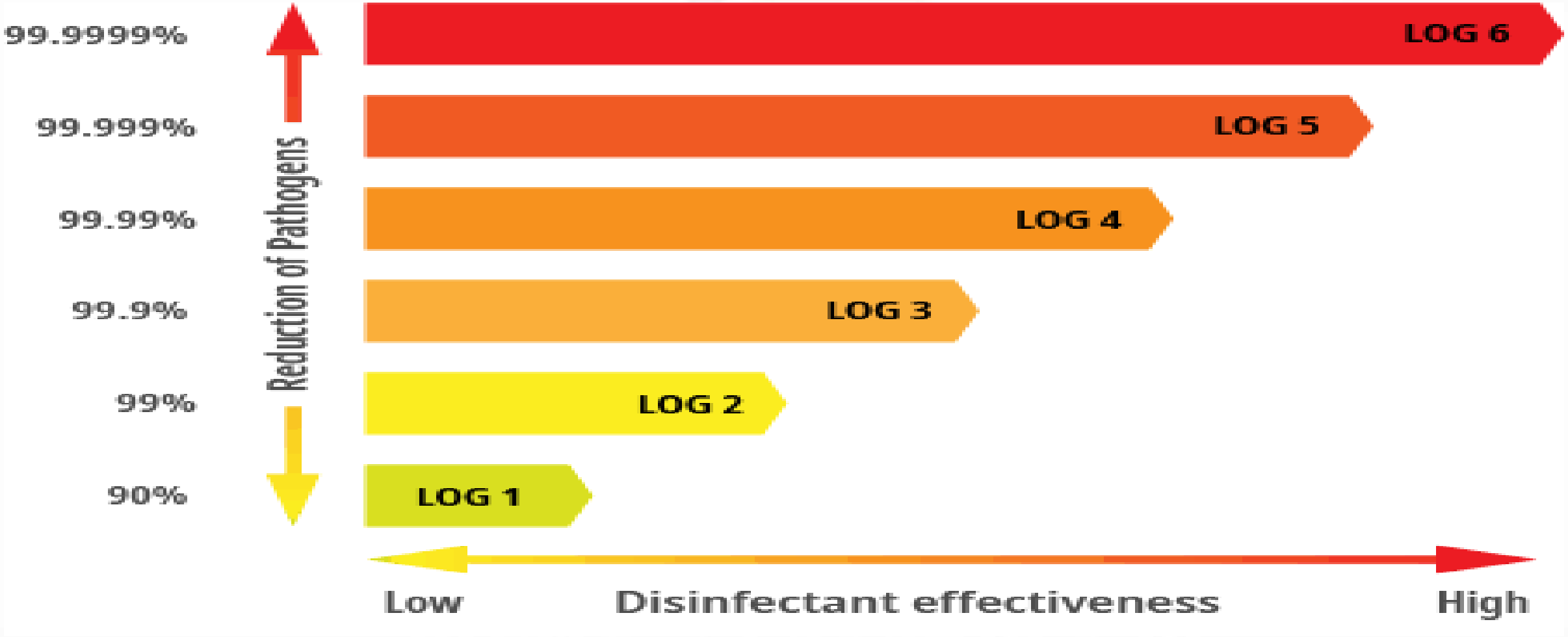
A is the number of viable microorganisms before treatment,

B is the number of viable microorganisms after treatment

Log Reduction Chart

Log Reduction	% Reduction of Bacteria
1	90
2	99
3	99.9
4	99.99
5	99.999

Percentage log reduction

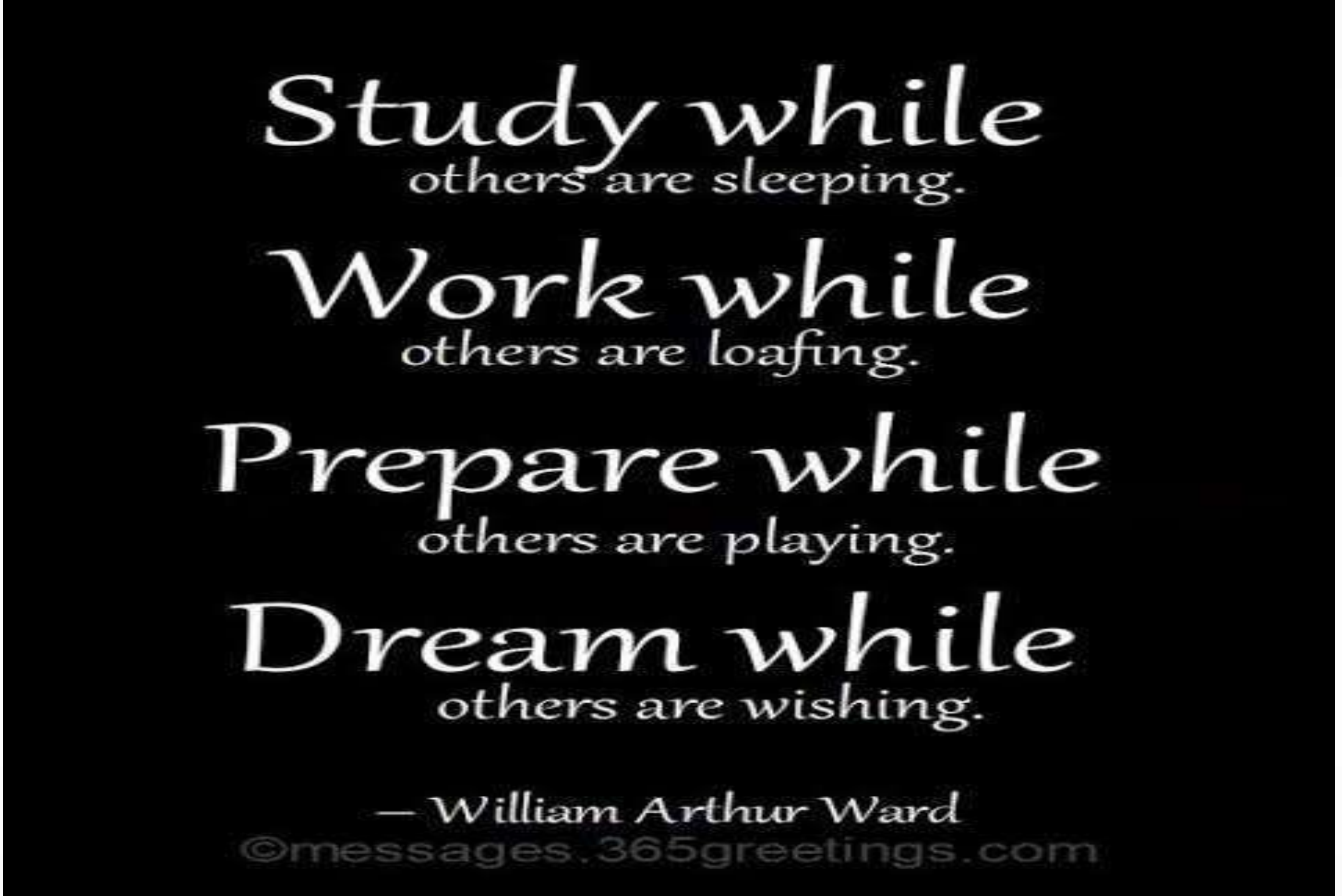


- Important Links: https://microchemlab.com/information/log-and-percent-reductions-microbiology-and-_antimicrobial-testing
- https://www.ciriscience.org/a_107-What-is-Log-Reduction

OTHER TECHNIQUES

- High sensitive analytical techniques are being investigate as possible replacement for the difficulty and time consuming pharmacopoeial tests.
- These include methods such as

1. **ATP bioluminescence**
2. **Electrical impedance spectrocopy**
3. **Spectro-fluorimetry**
4. **Chemiluminescence**


Study while
others are sleeping.
Work while
others are loafing.
Prepare while
others are playing.
Dream while
others are wishing.
— William Arthur Ward
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