- Robert Koch (1843-1910) could be considered the father of culture media.
- His first success in bacteriology was in the isolation of *Bacillus anthracis* which at the time was causing the disease anthrax in cattle.
- This was the first time that any pathogenic organism had been isolated and studied outside of the host's body.

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A satisfactory microbiological culture medium must contain available sources of:

- Carbon
- Nitrogen
- Inorganic phosphate and sulfur
- Trace metals
- Water
- Vitamins

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COMMON MEDIA CONSTITUENTS

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Cultivation of microorganisms depends on a number of important factors:

- Proper nutrients must be available.
- Oxygen or other gases must be available, as required.
- Moisture is necessary.
- The medium must have an appropriate pH.
- Proper temperature relations must prevail.
- The medium must be free of interfering bioburden.
- Contamination must be prevented.

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*A **growth medium** or **culture medium** is a **liquid** or **gel** designed to support the growth of **microorganisms** or cells, or small plants like the moss Physcomitrella patens. (***Madigan M, Martinko J (editors). (2005). *Brock Biology of Microorganisms* (11th ed.). Prentice Hall.**)*

Or

*Any **liquid** or **solid** preparation made specifically for the **growth, storage, or transport** of **microorganisms** or other types of cells.*

Liquid media

- Diffused growth
- No characteristics for identification
- Difficult to isolate
- Earliest liquid medium: urine or meat broth used by Louis Pasteur

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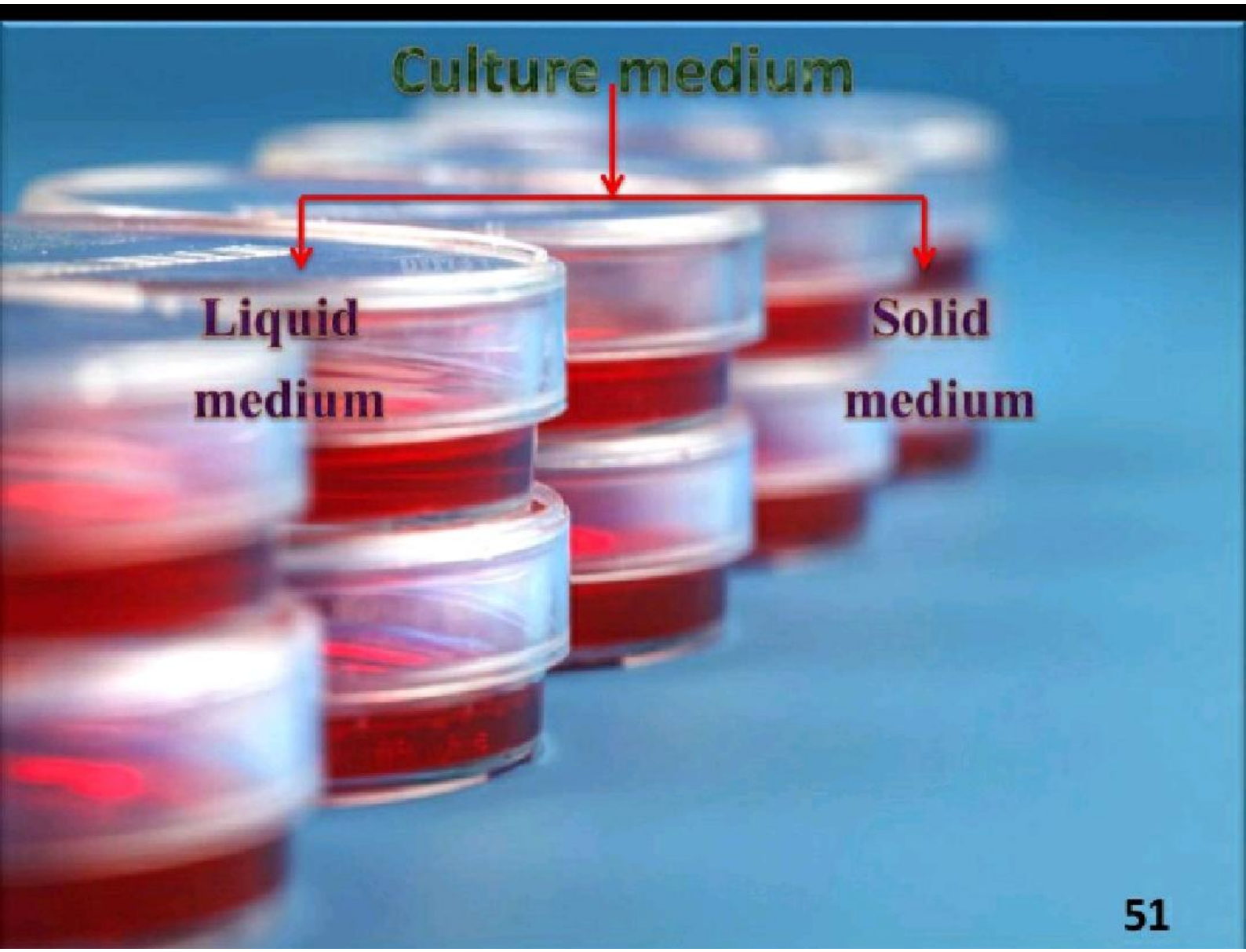
Culture medium
Liquid medium
Solid medium

Solid medium

- Distinct colony morphology
- Characteristics – easy to identify
- Colony – macroscopically visible collection of millions of bacteria originating from a single bacterial cell
- Earliest solid medium:

  Cooked cut potato by Robert Koch
- Gelatin - not satisfactory

  - liquefy at 24°C

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CONSTITUENTS	SOURCE
Amino-Nitrogen	Peptone, protein hydrolysate, infusions and extracts
Growth Factors	Blood, serum, yeast extract or vitamins, NAD (*Nicotinamide adenine dinucleotide*)
Energy Sources	Sugar, alcohols and carbohydrates
Buffer Salts	Phosphates, acetates and citrates
Mineral Salts and Metals	Phosphate, sulfate, magnesium, calcium, iron
Selective Agents	Chemicals, antimicrobials and dyes
Indicator Dyes	Phenol red, neutral red
Gelling agents	Agar, gelatin, alginate, silica gel

Special media

- Enriched media
- Enrichment media
- Selective media
- Indicator media
- Differential media
- Sugar media
- Transport media
- Media for biochemical reactions

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III.Based on Oxygen requirement

- Aerobic media
- Anaerobic media

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TYPES OF CULTURE MEDIA

I. Based on their consistency

a) Solid medium

b) Liquid medium

c) Semi solid medium

II. Based on the constituents/ ingredients

a) Simple medium

b) Complex medium

c) Synthetic or defined medium

d) Special media

Complex media

- Media other than basal media.
- They have added complex ingredients such as yeast extract or casein hydrolysate, which consist of a mixture of many chemical species in unknown proportions
- Provide special nutrients

Synthetic or defined media

- Media prepared from pure chemical substances
- Exact composition is known
- Used for special studies, eg. metabolic requirements
- Eg: peptone water- (1% peptone + 0.5% NaCl in water)

Simple media / basal media:

- Most common in routine diagnostic laboratories

  Eg: Nutrient Broth, Nutrient Agar
- NB consists of peptone, meat extract, NaCl, water
- NB + 0.5% Glucose = Glucose Broth
- NB + 2% agar = Nutrient agar
- Agar conc. Reduced (0.2 - 0.5%) = Semi-solid medium

Agar Plate
Agar Deep Tube
Broth
Agar Slant

Solid media – contains 2% agar

- Colony morphology, pigmentation, hemolysis can be appreciated.
- Eg: Nutrient agar, Blood agar

Liquid media – no agar.

- For inoculum preparation, Blood culture, continuous culture.
- Eg: Nutrient broth

Semi solid medium – 0.5% agar.

- Eg: Motility medium

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Enriched media

- Substances like blood, serum, egg are added to the basal medium.
- Used to grow bacteria that are exacting in their nutritional needs.
- Eg: Blood agar, Chocolate agar

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Enrichment media

- Liquid media used to isolate pathogens from a mixed culture.
- Stimulate growth of desired bacterium

  Inhibit growth of unwanted bacterium
- Media is incorporated with inhibitory substances to suppress the unwanted organism → increase in numbers of desired bacteria
- Eg: Selenite F Broth – for the isolation of *Salmonella*, *Shigella*

  Tetrathionate Broth – inhibit coliforms

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Differential media

- Substances incorporated in it enabling it to distinguish between bacteria.
- Eg: Mac Conkey's medium
  - Peptone
  - Lactose
  - Agar
  - Neutral red
  - Taurocholate
- Distinguish between lactose fermenters & non lactose fermenters.

Indicator media

- contain an indicator which changes its colour when a bacterium grows in them
- Eg:

  Wilson-blair medium – Salmonella Typhimurium forms black colonies

  Mcleod's medium (potassium tellurite)– Diphtheria Bacilli

  Christensen's urease medium

Transport media

- Media used for transporting the samples.
- Delicate organisms may not survive the time taken for transporting the specimen without a transport media.
- Eg:
  - Stuart's medium – non nutrient soft agar gel containing a reducing agent & charcoal used for *Gonnococci*
  - Buffered glycerol saline – enteric bacilli

CULTURE METHODS

- Culture methods employed depend on the purpose for which they are intended.
- Purposes:
  - To isolate bacteria in pure cultures.
  - To demonstrate their properties.
  - To obtain sufficient growth for the preparation of antigens and for other tests.
  - For bacteriophage & bacteriocin susceptibility.
  - To determine sensitivity to antibiotics.
  - To estimate viable counts.
  - Maintain stock cultures.

ANAEROBIC CULTURE METHODS

- Anaerobic bacteria differ in their requirement and sensitivity to oxygen.
- *Cl. tetani* is a strict anaerobe - grows at an oxygen tension < 2 mm Hg.

Methods:

- Production of vacuum
- Displacement of oxygen with other gases
- Chemical method
- Biological method
- Reduction of medium

Anaerobic media

- These media are used to grow anaerobic organisms.

  Eg: Robertson's cooked meat medium, Thioglycolate medium.

Temperature at $100^{0}C$

Boiling

Tyndallisation

2. RADIATION

Radiation sterilization: Ultra Violet rays are used to disinfect hospital operating rooms and safety cabinets.

- **Temperature below 100°C**

1.Vaccine bath

2. Inspissation

3. Pasteurization

~Low temperature holding method

~High temperature short time

Contd..

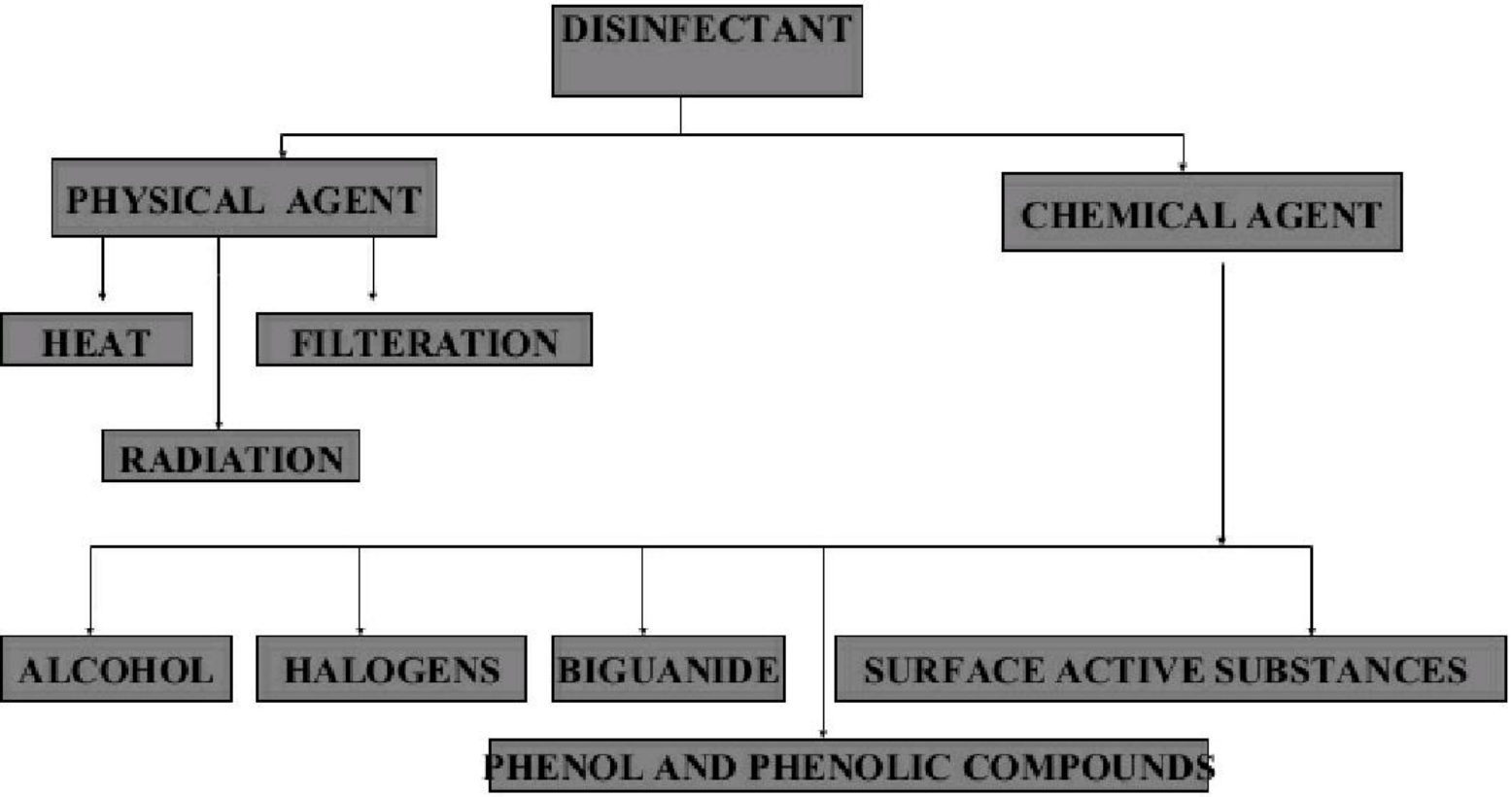
Uses:

a. **Separation of toxins and other soluble products of bacterial growth.**

b. **Sterilization of materials or other materials.**

c. **Purification of water.**

CLASSIFICATION OF DISINFECTION



TYPES OF FILTERS

I. CANDLE FILTERS

II. ASBESTOS FILTER

e.g. Seitz filter Carlson and sterimat filter

Disadvantages:

III. SINTERED GLASS FILTER

3. FILTRATION

- **It is the method of removal of bacteria from heat labile liquids such as sera and antibiotics solution.**

- **Factors affecting its efficiency are:**
  - **Initial number of microorganisms**
  - **type of microorganism**
  - **composition and pore size of the filter**

PHYSICAL AGENTS

1. HEAT

i. **Dry Heat**
   - a. Flaming

ii. **Moist Heat:-**
   - a. Temperature below 100^{0}C
   - b. Temperature at 100^{0}C
   - c. Steam at 100^{0}C

CHEMICAL METHODS

- **Many chemical agents are used as disinfectants and antiseptics**

II. CHEMICAL AGENTS

ALDEHYDES

a. Formaldehyde

b. Glutaraldehyde

ALCOHOLS

a. Ethyl alcohol

b. Isopropyl alcohol

c. Methyl alcohol

GENERAL RULES FOR USE OF DISINFECTANTS

1. **Disinfectants are generally toxic and may damage or irritate the skin, conjunctiva and mucosa. Care must be taken to avoid contact with irritant or toxic concentration. Disposable gloves should be worn when handling them.**

PHENOL AND PHENOLIC COMPOUNDS

Phenol and phenolic compound are effective disinfectants. Phenolic compound like bis-phenol *Hexachlorophane* has particular activity against Gram positive cocci.

FACTORS AFFECTING PERFORMANCE OF DISINFECTANTS

- Microbial load
- Characteristics of microorganisms
- Nature of exposed surfaces
- Presence of organic materials

iv. MEMBRANE FILTER

pore size range from 0.015-12micrometers

Uses: i. Preparation of solution for parenteral use.

ii. Disinfection of solution by removing bacteria and yeast.

v. SYRINGE FILTER

Uses: Disinfection of small volumes of fluids.

TYPES OF DISINFECTANTS

1. **HIGH LEVEL DISINFECTANTS**
   Examples: Aldehyde, gases
   Uses: for endoscope and surgical instruments

2. **INTERMEDIATE LEVEL DISINFECA NTS**
   Example: alcohol, iodophore
   Uses: laryngoscope

Contd..

Mode of action-

a. **Physical damage to cell membrane surface.**

b. **Precipitation of cell protein**

c. **Inactivation of enzyme**

C. BIGUANIDE

Chlorohexidine:

- ~ Less irritant and toxic to skin.
- ~ Commonly used as a skin disinfectant
- ~ Less active against Gram negative bacteria.

DISINFECTION POLICY

The policy should consider the following

- **The source for which the process is required.**
- **The processes and products available for disinfection.**
- **The category of process required for each item**
- **The specific product and method to be used for each item of equipment, the site of use and the staff responsible for the procedure.**

SURFACE ACTIVE AGENTS

These are substances that reduces surface tension.

Anionic, cationic, non-ionic and amphoteric

detergent are used generally.

1:16 PM

Chorea: Signs, Causes...
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More information

Overview

Chorea is a movement disorder that causes involuntary, unpredictable body movements.

Chorea symptoms can range from minor movements, such as fidgeting, to severe uncontrolled arm and leg movements. It can also interfere with:

- speech
- swallowing
- posture
- gait

ADVERTISEMENT

Agar

- **Fanny Hesse**
- Universally used for preparing solid medium
- Obtained from seaweed: *Gelidium*
- No nutritive value
- Not affected by the growth of the bacteria.
- Melts at 98°C & sets at 42°C
- 2% agar is employed in solid medium

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Selective media

- The inhibitory substance is added to a solid media
- Increase in number of colonies of desired bacterium

Eg:

- Desoxycholate citrate medium for dysentery bacilli
- TCBS – for *Vibro cholerae*
- LJ medium – *Mycobacterium tuberculosis*

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DEFINITIONS

- **DISINFECTION-** It is a process which reduces the number of contaminating microorganisms, particularly those liable to cause infection, to a level which is deemed no longer harmful to health

- **ANTISEPSIS-** It is used to describe disinfectants applied to living tissue such as wound.

contd.

3. LOW LEVEL DISINFECTANTS

Example: quaternary ammonium compounds
Uses: electrocardiogram, stethoscope

HALOGENS

i. Chlorine and Hypochlorite:

Both are widely used as disinfectants. Hypochlorites are inorganic chlorine containing compounds. Have bactericidal, fungicidal, virucidal and sporicidal action

ii. Iodine-

Used as skin disinfectant. Actively bactericidal but moderate action against spores.