Unit – 4

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Aseptic Area

asepsis → free from microbial contaminat^n.
sepsis → ____ " "

aseptic area

- an area where strict measures should be adopted or used to avoid contamination.
- To obtain this → aseptic techniques used → prevents microbial contamination into sterile products.

Designing of aseptic area

→ aseptic or sterile products → designed is → aseptic area → located seprately within industry/hospital.

Store Room	→ Prepⁿ area ↑ clean up area →	Aseptic filling area ↑↓ Sterilizatⁿ	Quarentine area ↓ Packaging & finishing	Storage & shipping

- Store room → to store ingredients
- Prepⁿ room → or compoundⁿ area → formulatⁿ of compounds/products done.
- clean up area → cleaning of crude drugs, raw materials done.
- Sterilizatⁿ area → compounded drugs are sterilized. to prevent microbial growth or to kill microbes.
- Aseptic filⁿ area → filling of compounds in container occurs.
- Quarentine area → Restricted area which runs under control of responsible person.

→ This area has a store where in process batches & approved batches of products are stored seprately.

- Packaging area → formulations packed here aseptically.
  → overall finishing is done.
- storage & shipping → as product formation completes & packaging done, they are stored in seprate aseptic area in controlled environment & at last shipping is done

Requirement for designing aseptic area

(1) site of place / premises

Aseptic area → away from stairs, lift & corridors.
→ Each stage of product done in seprate roo

(2) windows → large & transparent glass windows.
→ closed
→ ventilation provided by air filteration system.

(3) Doors → entrace (double door) with air lock system.
→ sliding doors used with self closing.

(4) floors, walls & bench tops
→ Easy to clean.
- smooth & no cracks or pores
- chemically resistant to solvents, acids or alkalis.

floor made up of —
a) Terrazzo → cement + marble
b) Linoleum → sheets or tiles
c) Plastics → PVC

Surface / tops of benches made up of :—
- Stainless steel
- Plastic laminates.

Laminar flow Eqpmnt

Laminar flow cabinet or laminar airflow hood is an enclosed bench → designed to → prevent contamination at time of :-

- Biochemical testing
- Performing oxn
- inoculating microbes (for pure culture)
- for obtaining clean / aseptic area.

Construction

Laminar flow cabinet consists of :-

- filter pad or pre-filter assembly
- A fan - Blower - HEPA filter
- Switches for (UV light, visible light & for motor)

Working

- Befor starting work on L.F.C
  UV germicidal lamp switched on for about 15 - 20 mins to kill germs
  ↓
  Then switched off as can cause skin burn or cancer
  ↓
  After this surface is wiped off with ethal befor & after use.

Principle

Fan sucks air → thru Pre filter assembly (dust trapped) → prefitered air passes by → HEPA filter with help of blower → Contaminating microbes were trapped → sterile air flows in cabinet.

HEPA (High Efficency Particulate Air) filter.

- most imp part of cabinet
- Pore size (0.3 μm) is size.
- filter medium in HEPA filter is made up of several foldings of fiberglass paper which are parallely arranged.

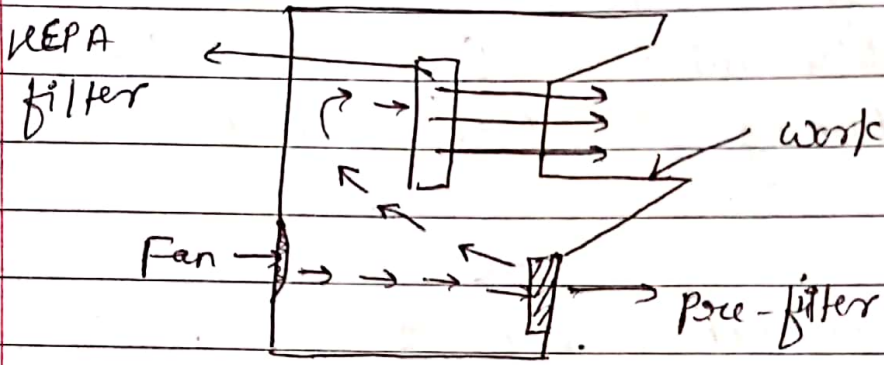
"Faith is the bird that feels the light when the dawn is still dark." - Rabindranath Tagor

Types of laminar flow cabinet

1. Horizontal laminar Hood
2. Vertical " "

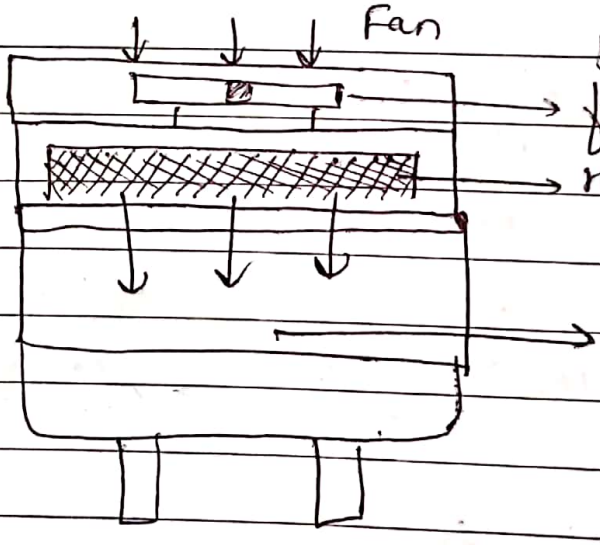
1. Horizontal laminar Hood

- These hoods filter air from back to front of the hood



2. Vertical L.H

- These hoods filter air from top to downward through the working area.



Diff. sources of contamination in an aseptic area

1. Personnel / Operator
2. Building
3. Equipment & Utensils
4. Raw materials
5. manufacturing process
6. HVAC (Heating, Ventilation & Air-conditioning) System

(1) Personnel

- Person = supervising, performing & controlling drug manufact. is reason for microbial contamin. due to follo. reasons:-
  - inadequate training — Improper hygine
  - Eating/drinking/smokn — Having any open wound/infection.

(2) Building

This is reason bcz of
- Rough floor, walls, ceilings — trce of moisture.
- Absence of air filteration systems
- inadequate lightining & ventilatn system.
- Improper washing, cleaning, toilet & personnel cleanliness.

(3) Equipment & Utensils

- Improper cleaning & sanitation due to comple design of equip.
- Using defective equipment.
- Corrosive Equipment

(4) Raw materials

- durgs = natural source = potential source of contamination:-
- Degradation due to extreme environmental conditions (heat, ck
- Wrong labelling
- Improper storage & Handling
- Incorrect sampling & testing

(5) Manufacturing process

- Improper sterilization — Exposing to open room environment.
- lack of labelling/cleaning

(6) HAVC

- Improper Air filteration system.
- Accumulation of organic material in system.
- non-maintenance of pressure in the area.

Method of prevention of Contamination

(1) Personnel
- Personal Hygine maintained
- Unauthorised personnel restricted.
- Trained persons allowed
- Should wear protective clothing, masks.

(2) facility design
- Pressure, & temp should be maintained in aseptic room.
- Air filteration system must be provided.
- Laminar flow hood must be fnt.
- Disinfectants are used to clean area.

(3) Building design
- Smooth, crack free & easily cleanable floors.
- Stainless steel sinks fnt.
- Windows & doors = closed properly.

(4) Cleaning & disinfection
- time to time cleaning
- good quality cleaning agents must be used.

no microbial & particulate ↑ controlling

Clean area classification

Black → dirty area
Grey → semi-clean area
White → clean
Is environment perfectly sterile, protective clothing

→ Clean room → Constructed → Conct^{n} of airborne → aseptic compounding is done.
is closed area where particles controlled by use of HEPA filter

→ Acc. to ISO (International Organisation of standardisation) → clean room refers → level of particulate cleanliness based on → no. of airborne particles of a certain size per cubic meter.

→ ↓ the classification no., cleaner is air.

- A clean room should include :-
  - HEPA filter
  - Air lock entry system
  - maintainance of + room air pressure
  -

Classificat^n of clean rooms & ppts.

Clean Rooms	ppts
(1) class 10000 (ISO class 7)	10000 or less particle of 0.5 μm & larger size exist in a given cubic foot of air.
(2) class 1000 (ISO class 6)	1000 or less particles of 0.5 μm in & larger size exist in a given cubic foot of air.
(3) class 100 (ISO class 5)	100 or less particles of 0.5 μm & larger size exist in a given cubic foot of air.

Microbial Assay.

Def → Method of examining the potency of activity of chemical comp. by use of micro-org.

Principle → Elaborated comparision of the "inhibition of growth" of the microbes by a measured concentration of antibiotics by known conc of standard with known activity.

Merits →
- simple & rapid
- Used for accurate standardisat^n of medicinal compound.
- Determines conc & activity of compound.
- Requires less amount of sample & instruments
- Suitable for compounds can't be assayed by physical or chemical methods

Demerits
- for particular assay, specific test org. req.
- Req. well trained & expert individuals.
- Sterile environment req.
- Invalic Result possibilites.

Standardization of Antibiotics

→ Antibiotics = kill, reduce & prevent microbial growth.

→ Two methods used:
(a) Cup plate or cylinder plate method
(b) Turbidimetric or tube assay "

(1) Cup plate / cylinder plate

* depends on diffusion of an antibiotic from a vertical cylinder or cavity thru solidified agar layer.

* Microbe growth → prevent → circular area (around cavity) → containing antibiotic.

Culture Media

Ingredients → - Peptone - Beef extract - NaCl
- Agar - Meat " - Dextrose

- Once culture media prepared → Sterilization done (autoclave)
↓
pH adjusted by NaOH or HCl.

Standard or Test soln prep.

- Stand antibiotic prep → dissolved/diluted → to produce req. concn.
in a solvent
- same way test soln prepared.

Antibiotic	Framycetin / Kanamycin	Test org
Chlortetracycline, Tetracycline, Tylosin, Tobramycin		Bacillus punilis Bacillus subtilis Stayphylococcus aureus. " "

* Test org → maintanid in culture media under incubation condth & weekly transfered to fresh media.

Method → Agar media prepared & sterelized (autoclave) 115°C for 30 min
↓
Transfered & poured in sterile plate & cooled & solidified.
↓
Test org. inoculated & spreaded on solidified agar med.
↓
sterile cavity / ditches / holes are made.
↓
Std & test antibiotic soln poured into cavities
↓
left to stand for 1-2 hrs
↓
Incubated at 32°C for 1-2 days.
↓
Microbial growth checked.

(b) Turbidimetric or tube assay method

- Shorter incubation period (4-5 hrs) for test org. growth
- Not used for cloudy or turbid prep.

Method :- Std soln prep. in 5 conc by diluting stock soln to make std curve.
↓
Medium concn selected & sample of test antibiotic soln diluted upto these concn.
↓
1 ml of each concn of std & sample soln is placed seprately in test tubes

In seprated test tubes, 9ml of nutrient media with test micro-org. is added

↓

3 control tubes prepared

↙ ↓ ↘

Culture control | blank control | Culture medium

↓

incubated (4-5 hrs)

↓

0.5ml of dil- formaldehyde soln added.

↓

absorbtiwity is checked in spectrophotometer at the wavelength of 530nm

↓

growth is determined

Microbiological assay of vitamin

Vit → Org. comp., an essential nutrient for org. in small quantity for proper functioning & metabolism.

(a) Vit B_{12} (cyano cobalamin) — Lactobacillus Leichmannii

(b) Vit B_7 (Biotin)
B_3 (Niacin)
B_5 (Pantothenate) → Lactobacillus plantarum.

(c) Vit B_6 (Pyridoxine)
B_2 (Riboflavin) → Tetrahymena thermophila.

* Test org → Lactobacillus Leichmannii, well grows in free of Vit B_{12}.

* Vit B_{12} → Sample Vit B_{12}
→ Standard Vit B_{12}.

* methods → ① Titrimetric
② Turbidimetric

"The butterfly counts not months but moments, and has time enough." -Rabindranath Tagor

Titrimetric

Clean test tube → 1ml, 1.5ml, 2ml, 2.5ml, 3ml & 3.5ml respectively of Std cyanocobalamin soln separately

↓

add basal medium (5ml)

↓

adjust final vol (10ml) with H_2O

↓

Another test tubes & take 1ml, 2ml, 3, 4 ml sep. test soln

↓

To each add 5ml basal medium & adjust vol (10ml) with H_2O

↓

now sterile all tubes in autoclave at 121°C for 15min

↓

cool & add test org

↓

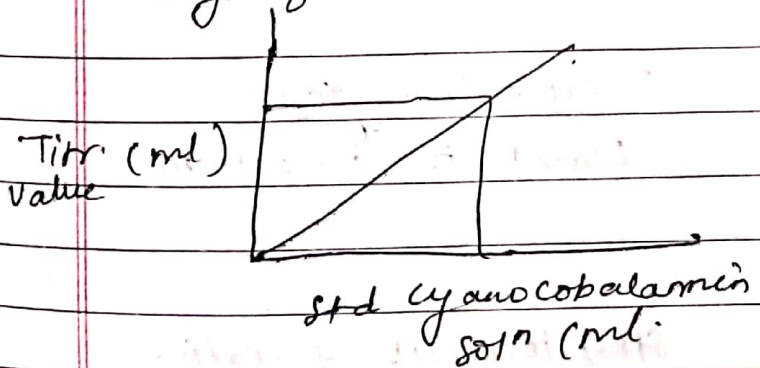
incubate at 30 - 37°C for 64 - 72hrs

↓

titrate contents of each tube with 0.5N NaOH using bromothymol blue indicator (converts to green)

↓

avg of titration value is determined & graph is potted.



Turbidimetric

- Clean test tube, add 1, 1.5, 2, 2.5, 3, 3.5ml separately std cyanocobalamin soln

↓

add basal medium (5ml)

↓

adjust final vol (10ml) with H2O

↓

Another test tube & add 1, 2, 3, 4 ml test sol^{n} seprately

↓

add 5ml basal med

↓

sterilize all tubes & cool it

↓

add test micro-org (Lact. Leichmannii)

↓

incubate at 30 – 37°C for 16 to 24 hrs.

↓

now spectrophotometer adjusted at wavelength of 580nm, transmittance is checked of each test tube.

Microbial Assay of Amino Acids

for assay, organisms used are –

	A. A	Test micro-organ
(a)	Alanine	Leuconostoc citrovorum
(b)	Arginine methionine Threonine Tryptophan	Streptococcus faecalis
(c)	arpartic acid cystine, glycine, histidine, Serine	Leuconostoc mesenteroides.

In a clear test tube, 5 concn of Std soln of AA is prep.

↓

Take other test tube, take medium conc. of Std & test sample soln was prepared

↓

Now prepare a basal media & add is all the test tubes of Std & test soln

↓

Test micro-org. is added is all the test tubes

↓

All of these were incubated for 18 to 24 hrs for 30–37°C

↓

Now the transmittance is checked by the help of spectrophotometer at 550 nm

Assessment of new antimicrobial agent is the process of discovery, evaluation and the Establishment of efficacy of identified synthetic or natural chemicals.

Identification means, finding of any chemical synthesized or obtained from natural sources like microbial cells or herbal / plant extracts.

Establishment of efficacy related with the findings that the new antimicrobial agent is effective against which organism, i.e. against bacteria (which strain), or fungi.

Establishment of effective concentration, means determination of concentration at which the chemical effectively kills the bacterial strains and also determination of Minimum Effective Concentration (MIC).

This method is most applied method for assessment of antimicrobial activity.

In this method agar plates are inoculated with any microbial cells (against which the antimicrobial activity is to be tested).

For this, the microbial cells are spread evenly (with the help of sterile cotton swab) on the surface of solidified nutrient agar media.

Then this seeded culture media is allowed to incubate in optimized conditions to allow the growth of microbial cells. After appropriate incubation, microbial cells formed a uniform layer over the surface of nutrient agar media (This is called Lawn Culture).