DESIGN OF ASEPTIC AREA

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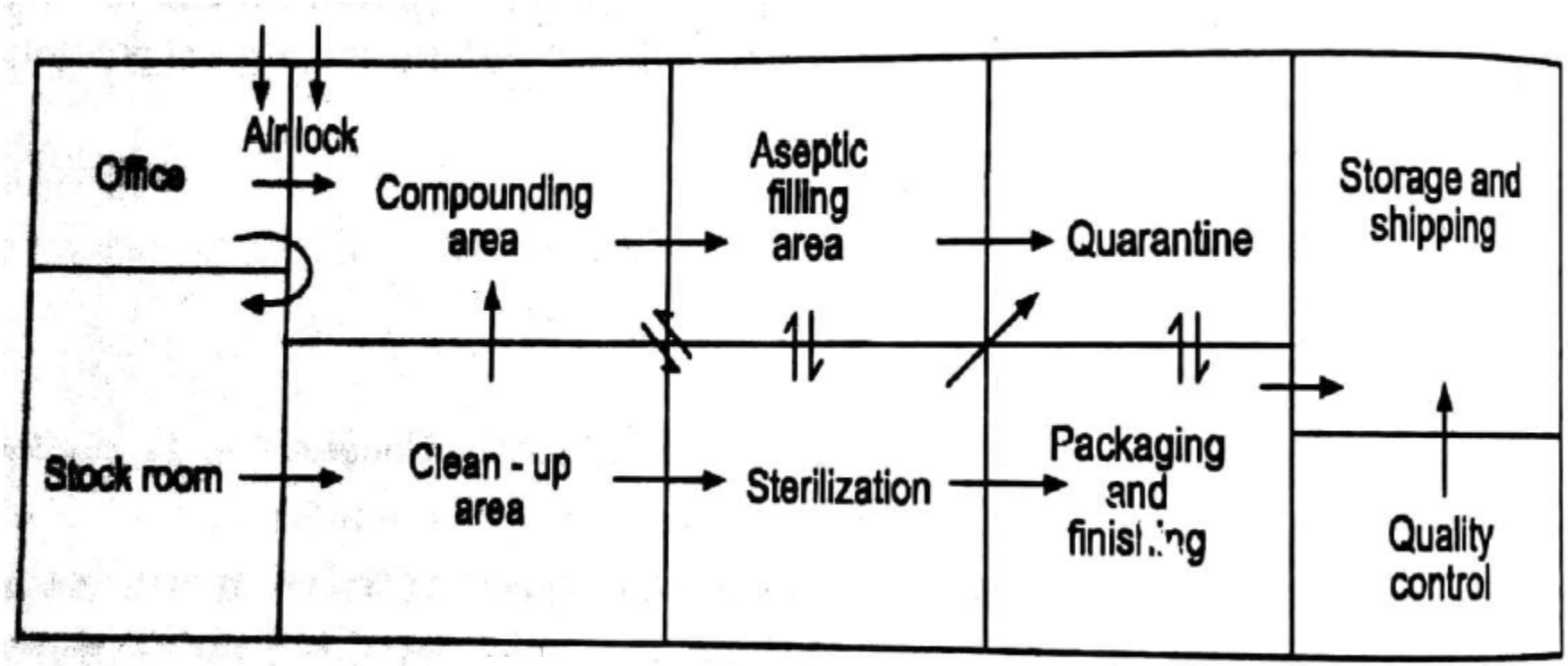
Building design, Construction and Production Facilities

- Production of sterile products should be carried out in a clean environment with a limit for the environmental quality of microbial and dust particle contamination.
- This limit for contamination is necessary to reduce the product contamination.
- The production area is normally divided into the clean-up area, the compounding area, the aseptic area, the quarantine area and the packaging area.

Flow diagram of aseptic area



Flow diagram of aseptic area

Floors, walls and ceilings

- All clean surfaces including the floor, walls and ceilings must be smooth, easy to clean, disinfected and be constructed to minimize microbial and particulate contamination.
- Flexing and non-flexing types of materials are used for construction of floor.
- **Flexing floor** materials are made up of synthetic elastromers of which most commonly used are polyvinylchloride (PVC). PVC flooring is easily repaired, cleaned, relatively cheap and simple.
- **Non-flexing floors** are made of hard inorganic filler substances in a matrix material. When a concrete is used it must be adequately sealed with a material resistant to chemicals, solvents and cleaning fluids.

Floors, walls and ceilings

- **Walls** must be made up of non-inflammable or fire resistant material e.g: Stainless steel, glass, enamelled steel etc.
- Generally plaster walls are easily damaged by the impact.
- For reduction of fungal growth , 1% of 8-hydroxyquinolone, pentachlorophenol etc may be added to the paint.
- Epoxy resin paints and polyurethane paints are also used to avoid cracking and peeling.
- The ceilings are sealed to prevent the entry of microbial contaminants.
- Internal fittings such as a cupboards, drawers, shelves, and equipments must be kept to a minimum.

Doors, windows and services

- Doors and windows should fit flush with the walls.
- Windows if required , are solely to provide illumination and are not for ventilation.
- Windows should be non-openable.
- Doors should be well fitted by maintaining the positive pressure air flow and self closing. Doors must be limited in number.
- **All pipes** passing through the walls of the room should be effectively sealed and should be flush fitting and easily cleaned.
- **Gas cylinders** should be excluded and all gases should be piped from outside the area.
- **Sinks and drains** must be excluded from the areas where aseptic procedures are performed in clean room areas.

Doors, windows and services

- **Light sources** in clean rooms are fitted with the ceilings to reduce the collection of the dust and to avoid the disturbance of the air flow pattern with in the room.
- **Non essential switches** such as room lighting switches should be installed outside the clean area.

Personnel and protective clothing

- The main source of contamination of clean areas arises from **skin scales** which are released by the operators.
- Personnel selected to work on the preparation of the parenteral products must be **neat and reliable.**
- They should be in **good health and free from dermatological conditions** that might increase the microbial load.
- Operator –borne contamination can be controlled by limiting the number of operators in clean area.
- All personnel should be trained for **good manufacturing practices and aseptic techniques.**

Personnel and protective clothing

- The operator should wear sterile protective clothing including **head wear, powder free rubber or plastic gloves, a non-fibre shedding facemask and footwear.**
- All protective clothing is designed to prevent the contamination from the body.
- All protective clothing must be sterilized by **moist heat sterilization or ethylene oxide sterilization.**
- Fresh sterile clothing should normally be provided each time the person enters the aseptic area.

Cleaning and disinfection

- Cleaning and disinfection procedures are used for the removal of microbial and particulate contamination.
- Cleaning agents are the alkaline detergents, non-ionic and ionic surfactants.
- Different types of disinfectants should be employed in rotation to prevent the development of resistant strains of microorganisms.
- Different concentration of quarternary ammonium compounds, sodium hypochloride, ethanol and formaldehyde solutions are used as disinfectants in cleaning area.
- Cetrimide or chlorhexidine in 70% alcohol are suitable for use as skin disinfectants.

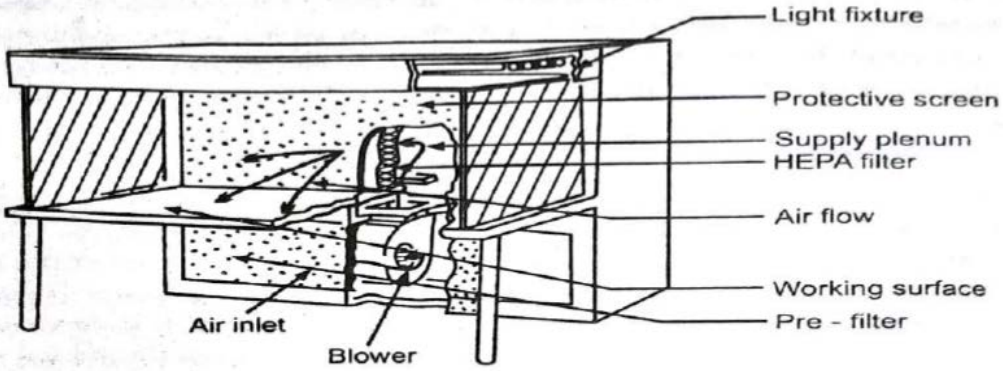
Air Supply

- The air supplied to a clean room must be filtered through **high efficiency particulate air (HEPA) filters.**
- The HEPA filter must be positioned at the inlet of the clean room and the pre-filter may be fitted upstream of the HEPA filters to prolong the life of final filter.
- HEPA filters are used in the construction of vertical and horizontal laminar air flow bench.
- The air filtered from the laminar air flow is claimed to be **99.97%** free from the microbial contamination.
- These filters are supported to provide **class 100 air** and they should be certified every 6 to 12 months.
- Air quality is evaluated using **settle plates, microbial air sampler or by particle counters.**

Laminar flow equipment

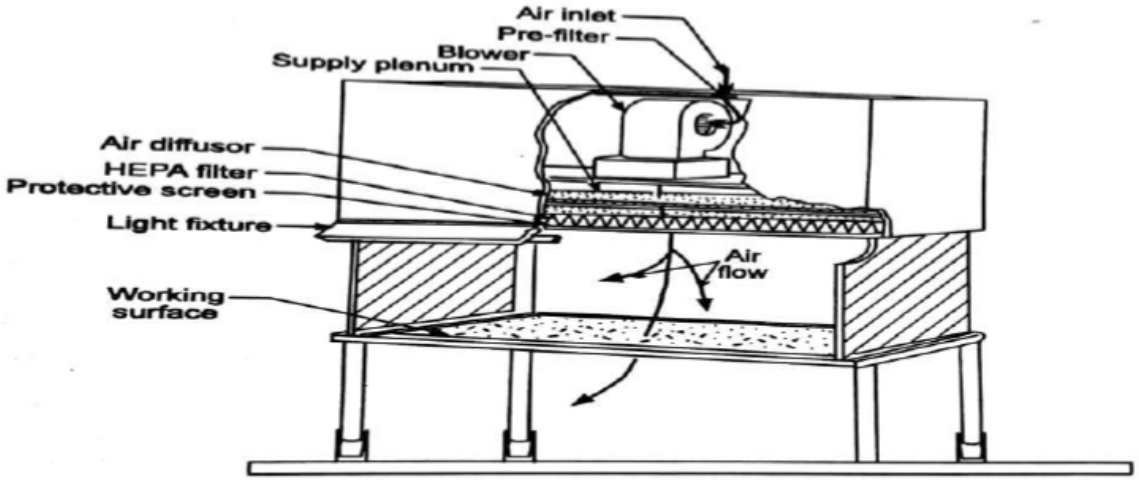
1. Vertical laminar air flow bench
2. Horizontal laminar air flow bench

Horizontal laminar air flow bench



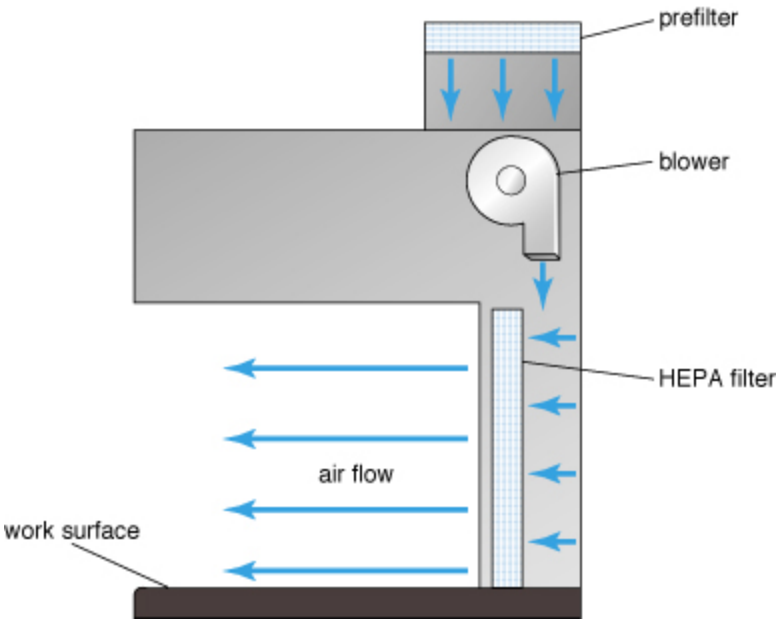
Horizontal laminar air flow bench

Vertical laminar air flow bench

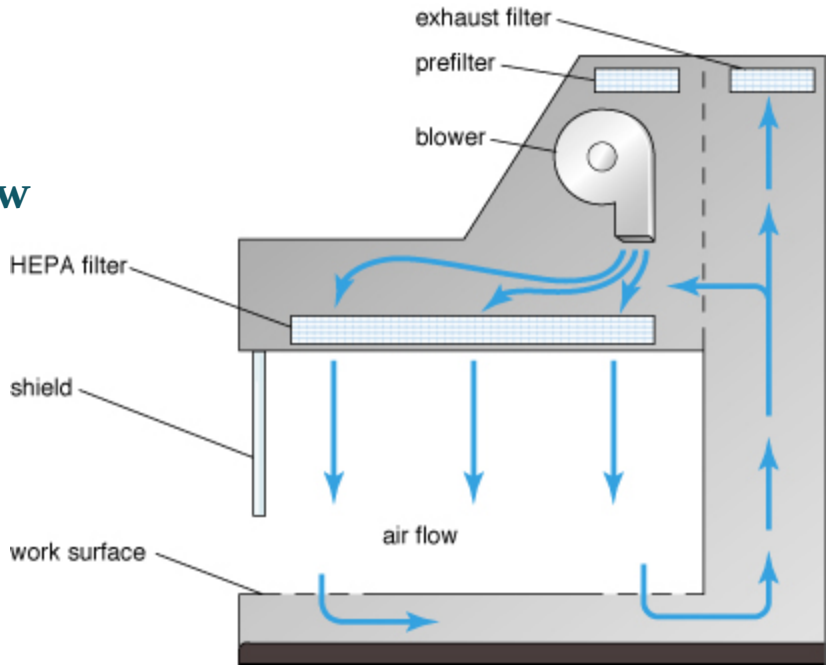


Vertical laminar air flow bench

Direction of Air Flow in Horizontal and Vertical Laminar Air Flow



Horizontal Laminar Air Flow



Vertical Laminar Air Flow

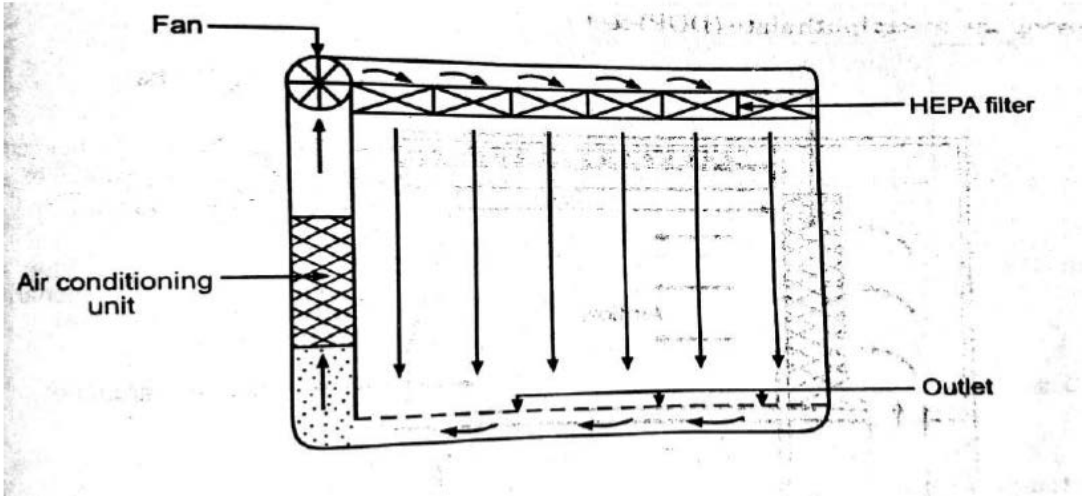
Air flow pattern

The air flow pattern within the clean room must be carefully regulated to avoid generating particles from the clean room floor, walls and operators.

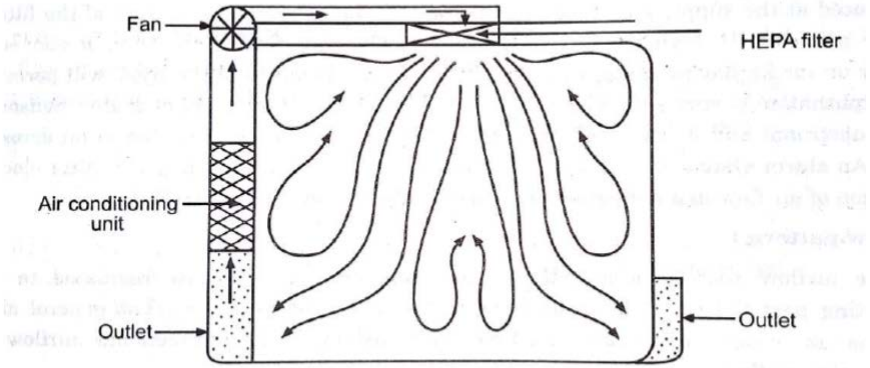
The general airflow patterns in Clean rooms are 3 types i,e

1. **Unidirectional airflow**
2. **Non-unidirectional airflow**
3. **Combined airflow**

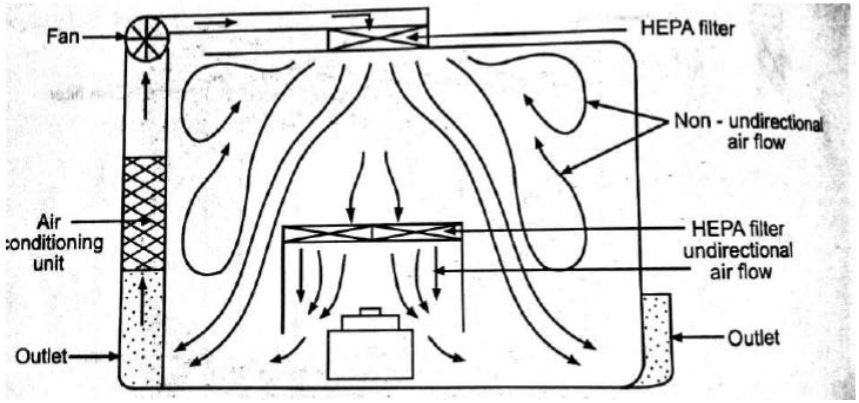
1. Unidirectional airflow



2. Non-unidirectional airflow



3. Combined airflow



Clean area classification

Grade	Maximum permitted number of particles /m³			
	At Rest		In Operation	
	0.5 µm	5 µm	0.5 µm	5 µm
A	3,520	20	3,520	20
B	3,520	29	352,000	2,900
C	352,000	2,900	3,520,000	29,000
D	3,520,000	29,000	Not defined	Not defined

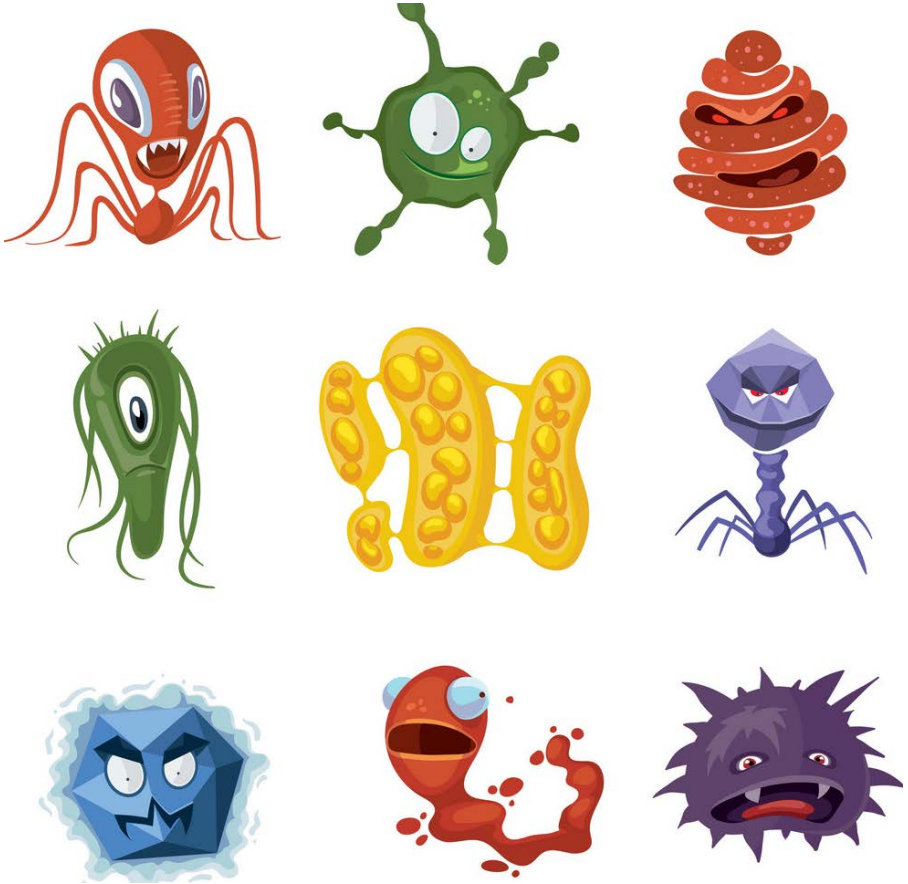
SOURCES OF CONTAMINATION

ASEPTIC AREA

- Asepsis is the practice to reduce or eliminate contaminants (such as bacteria, viruses, fungi, and parasites) from entering the operative field in surgery or medicine to prevent infection. Ideally, a field is "sterile" — free of contaminants — a situation that is difficult to attain.
- This area free from any contamination is ASEPTIC AREA

Sources of contamination in aseptic area and method to prevention

1. Atmosphere
2. Water
3. Raw material
4. Process operators
5. Equipment
6. Building
7. Packaging



ATMOSPHERE

- ATMOSPHERE IS UNABLE TO SUPPORT MICROBIAL GROWTH
- AIR MAY CONTAMINATE THE AREA
- OUTSIDE AIR
- INSIDE AIR
- DAMP AIR – LESS MICROORGANISMS

OPERATOR

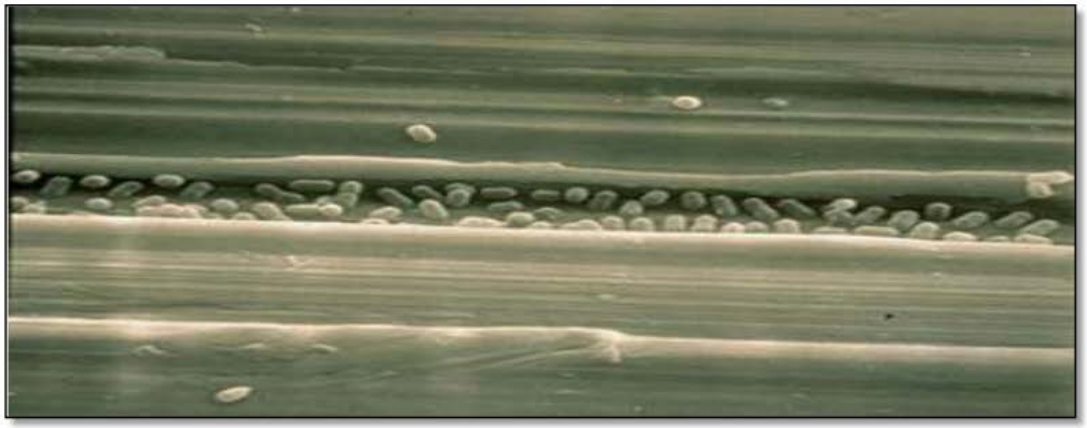
- SKIN,HAIR,CLOTHING
- IMPROPER HYGENE
- OPEN WOUNDS
- NASAL PASSAGES

RAW MATERIALS

- HIGHEST PROPORTION
- WATER

EQUIPMENTS

- WORKING SURFACES
- EXTERNAL SURFACES OF EQUIPMENTS



METHODS OF PREVENTION OF CONTAMINATION IN AN AREA

- DOORS, WINDOWS & SERVICES
- PROTECTIVE CLOTHING
- CLEANING AND DISINFECTION
- ENVIRONMENTAL CONTROL
- AIR SUPPLY

DOORS,WINDOWS & SERVICES

- NON OPENABLE WINDOWS
- TIGHT FITTING DOORS

- SELF CLOSING
- AIRLOCK
- EFFECTIVELY SEALED PIPES
- CEILING LIGHTS
- GAS CYLINDERS
- SINKS,DRAINS
- SWITCHES

PERSONAL & PROTECTIVE CLOTHING

- NEAT & RELAIBLE
- GOOD HEALTH
- LIMITED NUMBER OF OPERATORS
- RESTRICTED MOVEMENT
- WELL TRAINED
- PROTECTIVE CLOTHING
- ISOLATORS

CLEANING & DISINFECTION

- ALKALINE DETERGENTS
- IONIC AND NON-IONIC SURFACTANTS

ENVIRONMENTAL CONTROL

CLASS 10000 CLEAN ROOM

CLASS 100 CLEAN ROOM

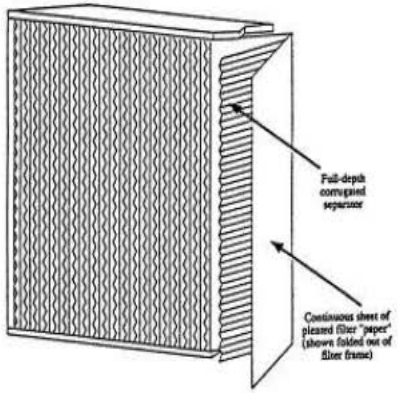
AIR SUPPLY

- HEPA FILTERS
- High Efficiency Particulate Air

Minimum particle collection efficiency:

99.97% for 0.3µm diameter particles

Filter made of pleated borosilicate glass



LAMINAR FLOW

- CAN DELIVER CLEAN AIR IN A VERTICAL/HORIZONTOL DIRECTION
- 99.9% FREE FROM MICROBIAL CONTAMINATION

TESTING OF CLEAN AND ASEPTIC ROOMS

AIR SAMPLING

WHY?

- collection of airborne microorganisms (in the context of microbiological assessment)

- **Air sampling** is a critical function of any Quality Control (QC) laboratory associated with a Pharmaceutical, Biotech, or healthcare facility.
- It also provides satisfactory conditions for proper processing of parenterals.

The methods of air sampling includes 3 types i,e:

- General methods
- Air sampling methods
- Surface sampling methods

General methods

- This method is used to validate HEPA filter, detect particulate contamination and monitor the environment.

It includes:

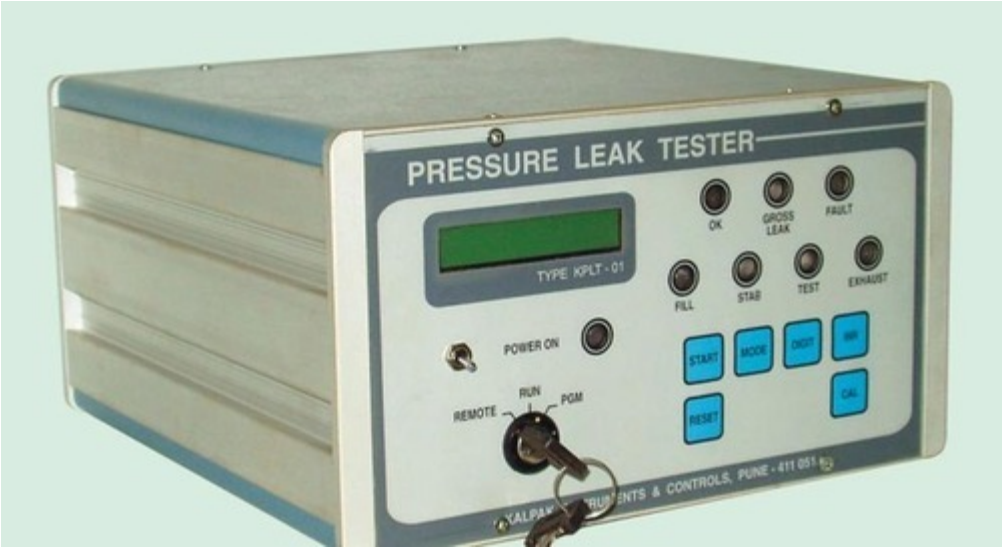
- **Filter efficiency test**
- **Induction leak test**
- **Particulate contamination control test**
- **Air pressure test**
- **Air flow test**
- **Noise level testing**
- **Lightning test**
- **Temperature and humidity test**

Particle Filtration Efficiency (PFE) Test

- The Particle Filtration Efficiency (PFE) test evaluates the nonviable particle retention or filtration efficiency of HEPA filtration devices at sub-micron levels. This test is performed on face masks and all filter material that allows 1 cubic foot per minute (CFM) flow to pass through it.

Induction leak test

When the atmospheric pressure is high, the amount of air per unit volume is high and when the atmospheric pressure is low, the amount of air per unit volume is low. Air flows from where the atmospheric pressure is high to where it is low and tries to maintain a balanced condition of atmospheric pressure.



Particulate contamination control test

- Clean benches are one type of mini clean environment and offer a convenient method of controlling particulate contamination within a controlled work area. They do not require a user to be gowned, but individuals will often wear gloves and arm covering when using the bench as an extra measure of protection against particulate contamination.

Air pressure test

The air pressure test is performed using Magnehelic Differential Pressure Gauges.

Air flow test

A **smoke** pattern **test** should be used to Various standards covering sterile operations including require an airflow visualization study to prove that the primary engineering controls (PEC) are suitable for the intended activity. The airflow must be unidirectional and devoid of turbulence and updrafts.

Noise level testing

- This test is designed specifically for measuring noise levels in the Laminar Air Flow work stations.

- Sound is vibration transmitted through a solid, liquid, or gas. Particularly, sound means those vibrations composed of frequencies capable of being detected by ears, and the intensity is commonly measured in decibel (dB).

- Prolonged exposure to loud sound intensity can lead to fatigue and distraction, which can be fatal during a contamination-sensitive work performed in biosafety cabinets, so it's important to measure the sound exposure.

Lighting test

- Lighting Measurement test Verifies the lighting level is meeting the LFH (**Laminar Flow Hood**) design level or worker comfort level.

temperature and relative humidity testing

- The temperature should be maintained at **21°C ± 3° C** inside the aseptic area all the time with corresponding relative humidity between **20 to 60%** though the ideal RH is considered to be **55%.** Appropriate devices to monitor and display these conditions inside the aseptic area may be installed.

Air sampling methods

- Electronic air particle counters
- Settle plates
- Slit air sample
- Liquid impinger
- Centrifugal air sample

What to be monitored?

- Non-viable airborne particulates
- Viable airborne particulates
- Viable surface bound particulates on cleanroom surfaces and personnel surfaces

Contamination Sources:

- People ~75%
- Ventilation ~15%
- Room Structure ~5%
- Equipment ~5%

Electronic air particle counters

(Measures non-viable airborne particles)

These are specially useful in determining the number of particles or microbes count per cubic feet to classify the cleanliness of particular room/area.

- A calibrated laser particle counter used to sample a defined volume of air.
- Can measure a variety of particle sizes, most commonly 0.5 and 5.0 micron.
- Particle counts are recorded as the number of particles per volume of air sampled.
- Results can be reported per cubic feet or **per cubic meter** as per ISO 14644.
- Handheld are ideal for spot checking.
- Important for tracking down a source of contamination, testing filters, and verifying the clean room is working within specified parameters.

Settling Plates
(Viable air borne particulates)

Settling plates filled with media are used to sample the microbial fallout over
time.

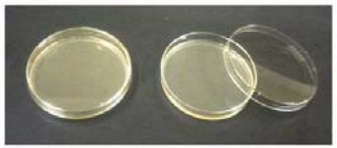
- The plates are incubated to promote growth
- Microorganisms are counted and results are reported as the number of CFU (colony forming units) per time sampled.
- In the absence of any kind of influence, airborne microorganisms, typically attached to larger particles, will deposit onto open culture plates.
- Microorganisms are usually found in the air of occupied rooms rafted onto skin cells. Very few present on their own.
- The average size of microbial particle will deposit, by gravity, onto surfaces at a rate of approximately 1 cm/s.

Contd.....

- Areas where there is little air movement (i.e. "dead spaces") or where airflows converge or are excessively turbulent. These conditions are most likely to occur:
- Adjacent to doors
- In pass through hatches
- At low level return air grilles
- Between HEPA's in clean rooms
- In corners of rooms
- Areas within the clean room where there is personnel activity or where specific operations are carried out.

Plates Before and After exposure

Settling Plate- Before Exposure



Settling Plate; After Exposure & After Incubation

Slit Air Sampler (Viable airborne particulates)

- Used to sample a defined volume of air, embedding viable particulates onto sterile media strips.
- It is the most widely used monitoring method for manufacturing of parenterals and quality control environments.
- In this method a known volume of air is directed into a plate of culture medium through a slit of 0.25 mm width, the plate is mechanically rotated in order to ensure uniform distribution of organism all over the plate.
- One cubic feet of air is allowed to pass through the slit and likewise 10 cubic feet of air is tested.
- The media strips are incubated to promote the growth of viable particulates
- The microorganisms are counted and results are reported as the number of CFU (colony forming units) per volume of air sampled.

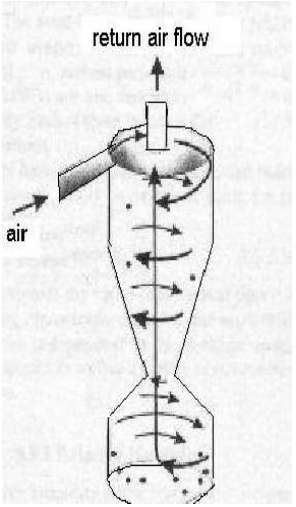
Liquid impinger

The air sample may be drawn into a measured volume of nutrient broth in an impinger.

Microorganisms in the broth then may be collected by membrane filtration , incubated and counted.

Centrifugal samplers

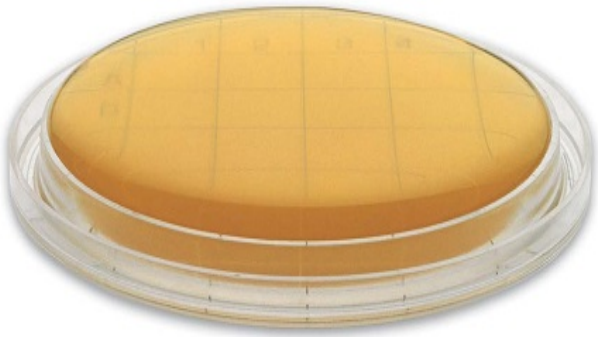
- Air is drawn into the sampler by an impeller housed inside an open shallow drum.
- The air is then accelerated by centrifugal force toward the inner wall of the drum containing agar medium onto which airborne particles are impacted.15 µm and larger particles.
- By applying centrifugal forces approx. 4000 to 4200 rpm the



Microbial particles are impacted at a high velocity onto the incubation

SURFACE SAMPLING METHODS

Rodac plates



- Samples or level of microorganisms on surface can be determined by specially build convex surface Petri plate.
- With rodac plates it is possible to roll the raised agar surface over flat or irregular surface to be tested.
- Surface contamination can be quantified by counting the colonies after incubation at 30 to 35°C for 48 hr.

Personnel gown monitoring

- RODAC plates are also used to monitor the contamination level of personnel gowns and Personal Protective Equipment (PPE) before or during manufacturing production.

Swab rinse test:

- The sterile cotton swabs are then placed into tubes of culture media & sample of water is placed on solid agar plate.
- This is a simple surface sample method employing sterile cotton swab to sample collection locations

AIR CLASSIFICATION SYSTEM FOR MANUFATURE OF STERILE PRODUCTS

	MAXIMUM NO.OF PARTICLES PERMITTED PER M_3		MAXIMUM NO.OF VIABLE MICROORGANISM PERMTTED PER M_3
GRADE	0.5-5 micron	Less than 5 micron	
A (Class 100) (Laminar-Airflow workstation)	3500	None	Less than 1
B (Class 100)	3500	None	Less than 5
C (Class 10000)	3,50,000	2,000	Less than 100

CONCLUSION

Clean and aseptic area as well as Air monitoring can ensure products are manufactured to the desired specifications.

Additionally, new technologies for air monitoring can help to detect contamination events faster and prevent future instances of contamination

THANK YOU