BACTERIOLOGY OF AIR

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Air Bacteriology

Introduction

- Air a mixture of gases
- Normal composition of external air by volume approximately-
  - Nitrogen -78%
  - Oxygen -20.93%
  - Carbon - dioxide -0.03%
  - Trace gases, trace elements

Air confined to a room undergoes chemical & physical changes:

- Physical changes-
  - Temperature rise
  - Increase humidity
  - Body odours
  - Decrease air movement

- Chemical changes-
  - Metabolic processes ↓ oxygen & ↑ CO_2

Definitions

Aerosol:

a mixture of tiny particles of fluids, gases & solids suspended in the air

Droplet: particles size ≥10μm diameter

Droplet nuclei: - tiny particles 1-10μm
- dried residues formed by evaporation of droplets coughed/sneezed
- generated by atomizing devices

Dust particles: larger droplets which settle down rapidly collecting dust

- Skin squames: ≥7.5 μm diameter shed at a rate of 6g/min

Particle Size

- Combustion particles - 0.01-0.05 μm to agglomerates

- Biological particles
  - get airborne from liquid or solid phase

- can be <0.5 μm ; usually >0.5 μm

Air born infection :

transmission of infection produced by respiratory droplets less than 5μm in size.

Droplet infection :

transmission of infection produced by respiratory droplets more than 5μm in size

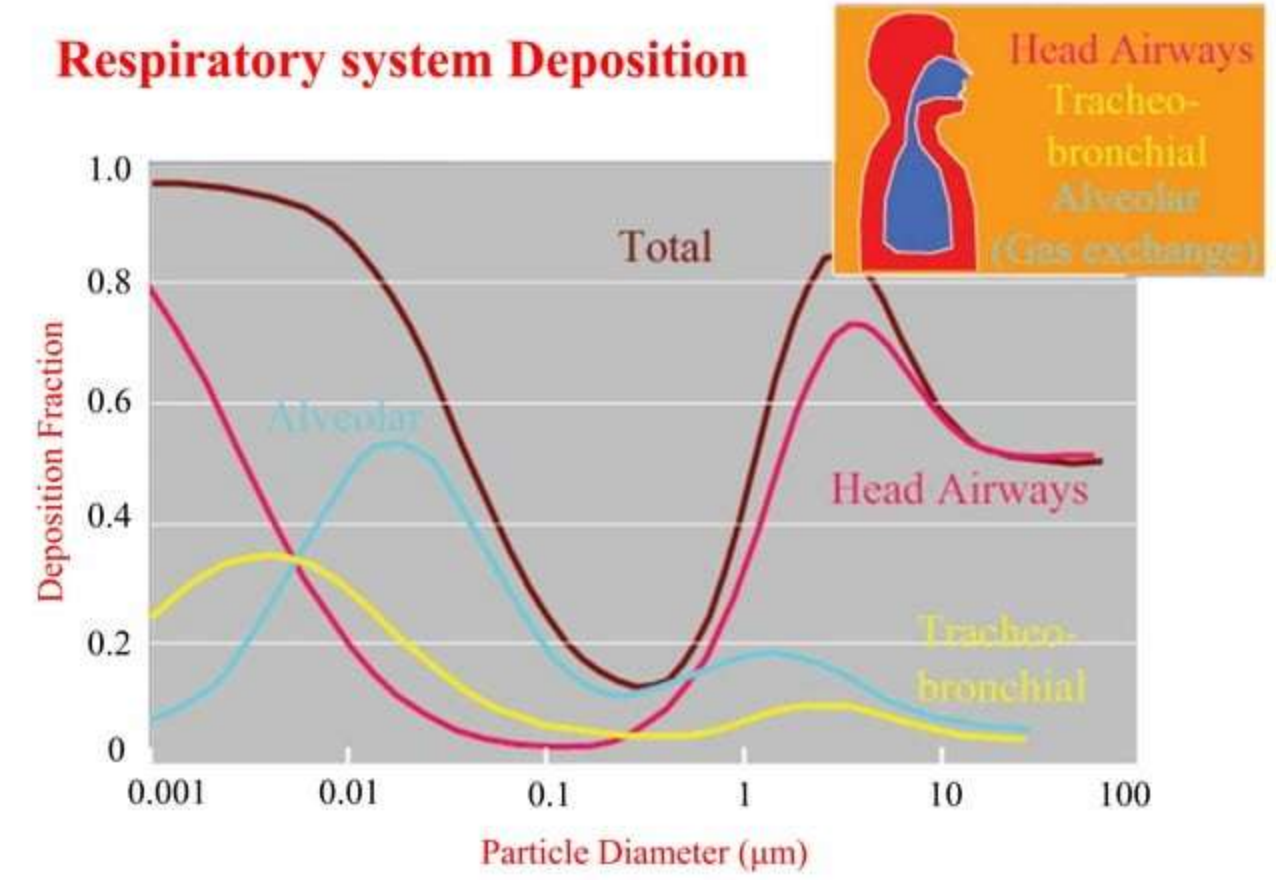
Features	Droplet transmission	Airborne transmission
1. Size of droplet	> 5 µm in size (droplet nuclei)	< 5 µm in size
2. Source of droplets	Produced during coughing, sneezing, talking, invasive procedures (e.g. bronchoscopy)	Produced during coughing, talking, sneezing, invasive procedures (bronchoscopy, suction aspiration)
3. Characteristics of droplets	• Droplet nuclei arise due to evaporation • Present in air for short time and travel only short distances (≤1 m) • Close contact needed for this mode of transmission	• Remain suspended in air for long periods • Travel several metres • Susceptible individual may become infected even if some distance from infected person
Microorganisms involved a) Bacteria	• *Streptococcus pyogenes* • *Neisseria meningitidis* • *Corynebacterium diphtheriae* • *Haemophilus influenzae* type B • *Bordetella pertussis* • *Yersinia pestis* (pneumonic plague) • *Mycoplasma pneumoniae*	*Mycobacterium tuberculosis*
b) Viruses	• Influenza viruses • Rubella virus • Mumps virus • Adenovirus • Parvovirus B19	Varicella-zoster virus Measles virus Influenza viruses

Note:

1) *S. aureus* and *S.pyogenes* are known to be shed and dispersed into air in operating rooms and newborn nurseries.
2) Outbreaks of pneumonia due to *Legionella pneumophila* are associated with the presence of cooling towers close to the ventilation systems of hospitals.
3) *Aspergillus* and other fungal spores dispersed through the air during construction, renovation and maintenance of buildings.
4) Although carpets, linen, potted plants and flowers are known to be reservoirs of opportunistic pathogens, epidemiological evidence linking these to nosocomial infections is lacking.

- Innumerable microorganisms are present all over in air in the environment.
- Apart from bacteria, molds and viruses are also present and can be transmitted from person to person in the form of aerosols.
- The proportion of the dust particles or aerosols reaching the lung depends on their size.
- All particles over 5μm are retained in the nose and
- those of 5 μm reach the lung and are retained in the alveoli.

Respiratory system Deposition



Bacteria in Air

The bacterial content of air- depends on the location, i.e., whether it is outdoor air or indoor air.

Outdoor air -

- Human & animal pollution
- Nature of soil
- Amount of vegetation
- Atmospheric conditions
  - Humidity & rainfall
  - Temperature
  - Wind
  - Sunlight

Indoor air-

- The number of bacteria in the air at any time is dependent on a variety of factors:
  - the number of persons present,
  - the amount of their body movements and
  - the amount of disturbance of their clothing.

  - ➢Droplets & droplet nuclei
  - ➢Dust
  - ➢Skin squames

- The ultimate source of common pathogenic organisms is dust, derived from human beings.

Bacteria in Air

- Spores and fragments of moulds are more numerous than bacteria.
- Bacteria in the upper air consists largely of aerobic spore-bearing bacilli and to a much less extent Achromobacter, Sarcinia and Micrococcus.
- Pathogens seldom survive the adverse conditions of the outdoor air to cause disease.
- Pathogenic bacteria do not multiply in air.

Bacteria in Air

Laboratory aerosols are generated by -

- Heating loop directly after inoculating
- Centrifugation
- Pipetting
- Pouring of liquid cultures
- Mixing a fluid culture with a pipette
- Breaking of tubes of broth culture
- Splashes

Transmission

Airborne transmission describes organisms having true airborne phase in their route of dissemination.

- Microbial sources –

  - Infectious patient - open case of pulmonary TB

  - Metabolic/decomposition product
    - moldy hay hypersensitive pneumonitis

  - Environmental niche - *Legionella* from humidifier water

Airborne Agents

Organisms implicated –

- *Mycobacteria*
- *Staphylococcus*
- *Streptococcus*
- *Bacillus*
- *Nocardia*
- *Actinomycetes*
- *Pseudomonas*
- *Burkholderia*
- *Klebsiella*
- *Escherichia*
- *Proteus*

Airborne transmitted infections

Community acquired infections

- Outbreak amongst 100 school children following exposure to a guard who was an open case of pulmonary TB for 6 months

- School bus driver transmitted TB to the children traveling in the bus

Hospital acquired infections

- Care giver who is suffering from lower respiratory tract infection, transmitted the infection to the patient

- Nurse in an OT suffering from Staphylococcal infection on the face transmitted the same to a patient leading to wound infection

Airborne transmitted infections

Hospital acquired infections

- In OT major source of contamination is the surgical team
- A person releases about 10 million particles/day from his/her body
- The release rate is 10,000 particles per min while walking alone
- About 5 - 10% of these particles carry bacteria
- Patient is not usually a source of significant contamination as his/her movements are minimal

Sick building syndrome

- Also known as Building Related Illness

- Defined by WHO- excessive reporting by the building occupants of
  - headache
  - fatigue
  - nasal congestion
  - eye irritation

- Appears rapidly 2-6 hrs after entering the building
- Syndrome is temporary
- Disappears within few hours after exiting
- Related to air supply system in air tight, moist/humid building
- Mostly due to certain bacteria & fungi thriving indoor

Epidemiological Factors

Release of microbes from contaminated surfaces

- Air velocity increased
- Colony structure-dry, friable
- Moisture conditions-increased
- Vibration
- Rapidity of release

Agent factors

- Ability to survive harsh environment
- Infectivity
- Resistance to disinfection
- Ease of spread

Epidemiological Factors

Host factors
- Immunocompromised host
- Hospital admission, increased duration of hospital stay

Care-givers
- Ill care-giver – respiratory infections
- Breach in practice of standard operative protocols
- Lack of awareness

Control Strategies

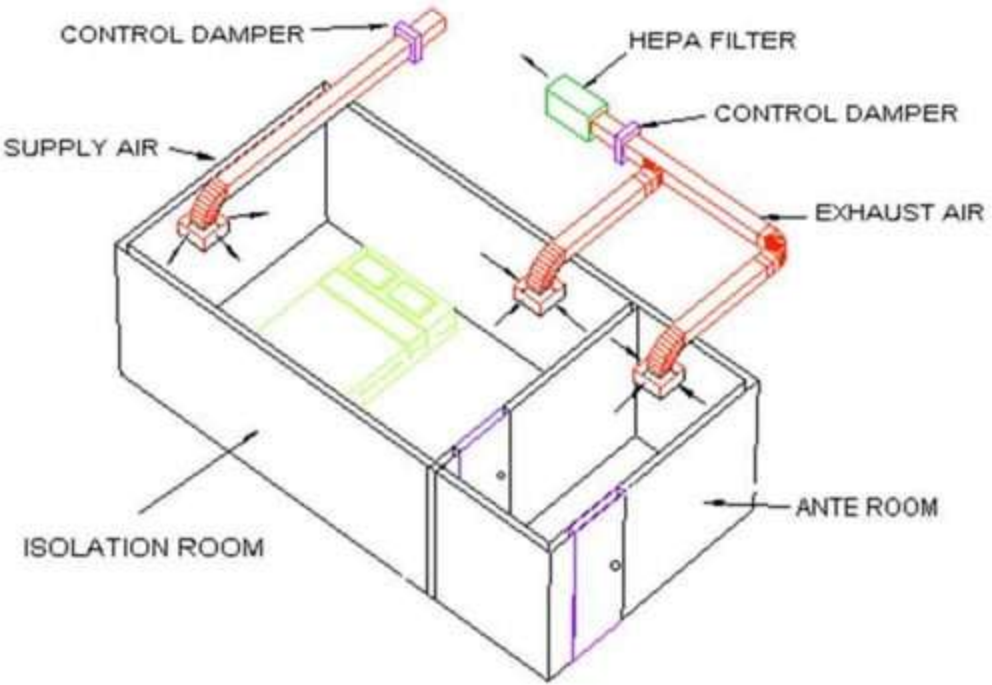
Current strategies -

- Isolation systems
- Air filtration
- UV irradiation
- Fogging/fumigation
- Engineering control

Future strategies -

- Air ozonization
- Photo catalytic oxidation

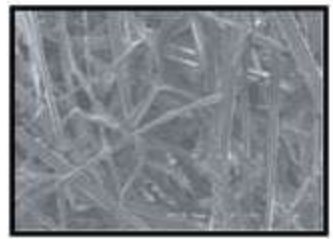
Isolation system



Control Strategies

- Air filtration
  - Essential element of ventilation systems - controls contaminants

- HEPA Filter - work efficiency of 99.7 % to 99.97 %
  - traps particles - 0.05 µm to 0.15 µm
  - extremely effective



- ULPA Filter – work efficiency of 99.997 % to 99.999 %
  - extremely low levels non-viable particulate contamination

Most studies found HEPA Filter better than ULPA Filter.

Particle Collection

Collection mechanisms govern particulate air filter performance –

Interception

- Trapping by contact of microbe with filter surface
- Small diameter of filter matrix favorable

Sedimentation

- Depends on settling rate of microorganism

Impaction

- Momentum of microorganism traveling in a gas stream
- Collides with fiber surface
- High gas velocity & small fiber diameter of filter matrix

Particle Collection

Diffusion

- Low velocity is favorable
- Brownian movement contacts bacterium to fiber

Electrostatic

- Gas flow through filter matrix results in charging of filter fibers
- Charge acquired depends on nature of fiber
- This charge attracts microorganism
- Hydrophobicity is an important characteristic of air filters
- Filters must not be moist - charge is dissipated

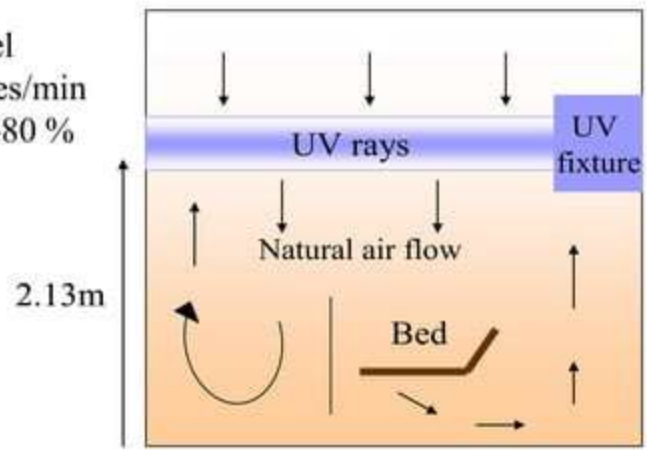
UV Irradiation

- UV kills microbes by damaging DNA

- Lethe – amount of UV necessary to kill bacteria in one turn over air
  - expressed in Watt – minutes / sq foot

Methods of irradiation

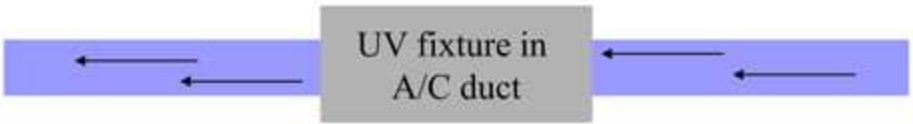
1. Upper air irradiation
   - Lamps placed above eye level
   - Requires 1-3 room air changes/min
   - UV reflectance enhanced 40-80 % by aluminum metal & paint



UV Irradiation

Methods of irradiation

2. UV lamp without reflectors in heating and A/C ducts.
   - Arrangement of lamp is perpendicular to flow of air
   - Lamps are best installed near filters where air velocity is minimum.
   - UV room A/C maintains clean air, temperature & relative humidity.



3. Irradiation of air in enclosed space by unshielded lamps.
   - Used in cabinets for storage of life saving equipments
   - Narrow intensive curtains of UV across entrance to critical wards

CDC recommends use of both mechanical ventilation & UV irradiation

Fogging & Fumigation

Hydrogen peroxide

- Damages bacteria by denaturing DNA, membrane lipids & cell components
- Vapor phase causes rapid spore inactivation
- Penetrates plastics - polypropylene, PVC, polyethylene
- Does not require pressurized chamber
- Can be used for fogging at 4°C
- Residual effect is good
- Not carcinogenic

Fogging & Fumigation

Formaldehyde

- Good sporicidal, bactericidal action
- Proven carcinogen, skin irritant, leads to bronchial asthma
- Residual effect poor
- Being phased out (no longer recommended)

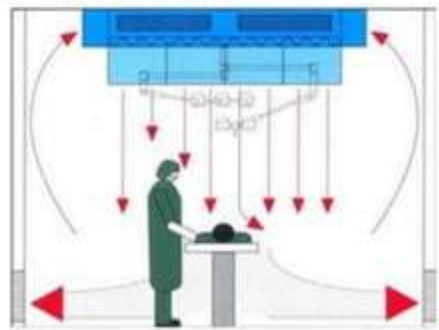
Procedure of fumigation -

- 500ml of formalin/100ft^3
- Seal the room
- Ensure everything is dry
- Add KMn04 10g/35ml
- Wait for 24-48 hrs
- Neutralize with conc. Ammonia day after

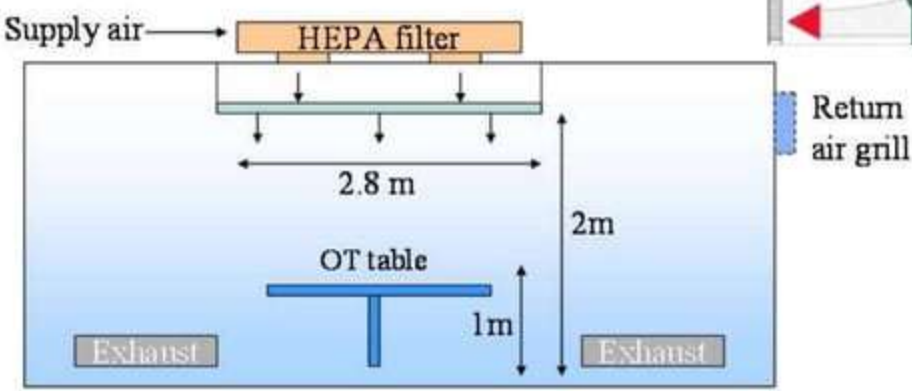
Engineering Control

- Laminar air flow - unidirectional / linear air flow
- Turbulent air flow - causes increase dispersion of microbial particles
- Convection currents due to heat/movements - velocity 0.46 m/sec

- Ventilation modification
- Conditioning of air



Ultra clean ventilation system



Future Strategies

Ozonization –

- Ozone is injected into the air stream & mixed in a turbulator
- all organic compounds, including viral nucleic acids & bacteria destroyed
- corrosive action

Photo catalytic oxidation –

- Titanium dioxide (TiO_2) is a semiconductor photo catalyst
- On irradiation releases hydroxyl ion
- Kill and decompose adsorbed bioaerosols
- Candidate for indoor air quality (IAQ) applications

Respirators

Strict biosafety precautions to be followed (eg.TB)

N95

Types of respirators available:

Non oil-proof -

N95 - Filters ~ 95% of airborne particles.
N99 - Filters ~ 99% of airborne particles.
N100 - Filters ~ 99.97% of airborne particles.

Three layered mask

Relatively oil-proof -

R95 - Filters ~ 95% of airborne particles.
R99 - Filters ~ 99% of airborne particles.
R100 - Filters ~ 99.97% of airborne particles.

Oil Proof -

P95 - Filters ~ 95% of airborne particles.
P99 - Filters ~ 99% of airborne particles.
P100 - Filters ~ 99.97% of airborne particles.

Air Sampling

Purpose - measure microbial contamination in air

Types:

Passive air sampling -

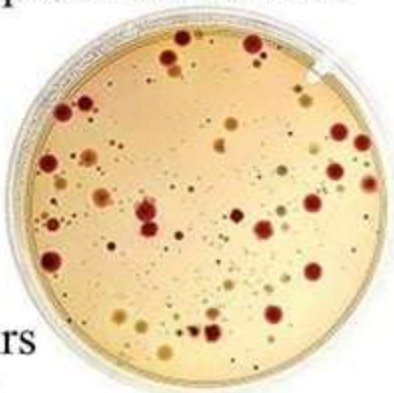
- settle plate method

Active air sampling -

- Slit-type Impactors
- Sieve-type Impactors
- Centrifugal air samplers
- Filtration samplers
- Cascade impaction
- Electrostatic precipitation

Settle Plate Method

- Open plates of culture media exposed to room air -
  - Nutrient media - BA, TSA
  - 1m height above floor
  - 1m away from the wall
  - for 1 hour.



- Plates incubated at 37°C × 24 hrs
  → number of colonies counted.

- Large bacteria-carrying dust particles settle on the medium.

- Gives an idea of relative no. & type of organisms- count of the colonies formed shows the number of settled particles that contained bacteria capable of growth on the medium
- The colonies are counted, preferably with the use of a plate microscope to detect the smallest ones.
- Used for testing surgical theater and hospital ward air
- The result is expressed as the number of bacteria-carrying particles settling on a given area in a given period of time.

- The method has the advantage of simplicity, but measures only the rate of deposition of large particles from the air, not the total number of large and small bacteria-carrying particles suspended in it.

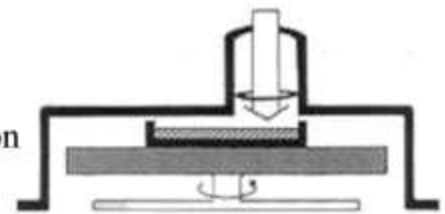
Slit Sampler

Slit sampling machine is

- ➢The most efficient and convenient device for counting the bacteria-carrying particles suspended in a unit volume of air.
- ➢a device for drawing a measured quantity of air from the environment.

Known vol. of air is directed onto a plate through a slit 0.25 mm wide

- Plate being mechanically rotated
  organism is evenly distributed
- Valuable in areas of low microbial contamination
- Microbial content of compressed air –
  - assessed by bubbling known vol. through a nutrient broth
  - then filtered through a membrane.
  - membrane incubated on nutrient agar & viable count done



- The efficiency of collection, even for the smallest bacterial particles, is very high.
- Disadvantages of the slit sampler: noisy and relatively cumbersome.

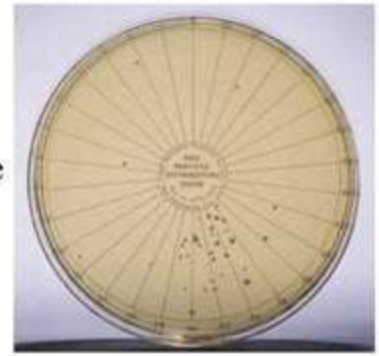
Slit Sampler

Formula for calculating microbial growth by slit – sampler -

Bioload (B) - Level of bacterial contamination of air expressed as the number of bacteria carrying particles per m^3

$$B = \frac{1000\ n}{RT} \quad bcp/m^3$$

n = number of colonies counted on the sample plate
T = duration of the test in minutes
R = air sampling rate in liters/min.



Cascade Impaction Sampler

- Lidwell (1959) - described the impaction sampler
- Operates on the cascade principle & collects airborne infected particles in four ranges of size on four separate culture plates
- The size ranges of particles are -
  - <4 microns
  - 4 - 10 microns
  - 10 - 18 microns
  - >18 microns.
- Majority of bacteria in air are 10-18 microns

Deposit spikes

Air Sampling

Centrifugal air sampler -

- Wells (1933) - sampled air passed along a plastic tube lined with nutrient agar passed along its side
- Resembles a cylindrical torch having a drum
- Air is drawn into drum, suspended particles impact on the medium
- Less efficient than slit sampler

Electrostatic precipitation -

- Efficient method
- Low resistance of moving particles enables high flow rates
- Reduced power for the provision of suction

Active Air Sampling

Advantages

- Most official guidelines refer to cfu/m^3
- Sample collection is rapid

Disadvantages

- Device difficult to sterilize
- Expensive
- Noisy
- Different samples give different results
- The same sampler gives different results
- Fallout of microorganisms is not evaluated
- Sampler must be frequently calibrated
- The air exhaust must be removed
- The airflow is disturbed
- Some microbes are inactivated by the impact on the nutrient

Passive Air Sampling

Advantages

- Cheap
- Available everywhere
- Sterile
- Multiple samples simultaneously in different places
- Meaningful samples (for the contamination of critical surface)
- Reliable results
- Comparable & generally valid results
- Airflow is not disturbed
- Reproduce real conditions

Disadvantages

- Not always accepted by official guidelines

Dust Sampling

Sweep plate

- Personal clothing, linen, curtains contain dust laden bacteria
- Culture plate swept over the surface, dust settles on the medium
- Colonies are identified & counted

Dust sampling

- sterile moistened cotton wool swabs
- collect dust from surfaces, floors
- Swabs inoculated onto suitable media
- colonies identified & counted
- Routinely employed in OT

Recommended counts

Active air sampler -

- Conventional OT – up to 180 cfu/m^3
- Ultra clean ventilated OT - <10 cfu/m^3 acceptable
- ICU-<50cfu/m^3

Passive air sampler -

- No recommended air counts
- Variation in plate diameters
- Variation in time, usually for 15min-1hr

European union comparison study on bacterial counts:

Group	Active air sampler	Passive air sampler (90mm plate)
A	<1cfu/cubic m	<1cfu/plate
B	10cfu/cubic m	5cfu/plate
C	100cfu/cubic m	50cfu/plate
D	200cfu/cubic m	100cfu/plate

Quality of Air

Described by maximum level of contamination permitted

- US F209 recognized 6 classes
- European union recognized 4 grades

US	EU	0.5μm particles
1	-	1/ft^3 (35/m^3)
10	-	10/ft^3 (350/m^3)
100	A	100/ft^3 (3500/m^3)
1000	B	1000/ft^3 (35000/m^3)
10000	C	10000/ft^3 (350000/m^3)
100000	D	100000 /ft^3 (3500000/m^3)

Surveillance

No standard guidelines available

Recommendations of HIS for OTs

- Engineering control
  - excellent ventilation performance
  - no architectural compromise
- Administrative control
  - training of entire staff
  - restricted traffic
- Surveillance
  - before commissioning
  - after any construction/maintenance
  - should be annually done in UCV- OTs

Surveillance

Critical care units - No statutory recommendations available

Engineering control
- individualized cubicle
- isolation rooms available
- adequate space around each bed
- traffic circuits for clean & dirty equipment segregated

Administrative control
- CME for entire staff
- written protocols

Surveillance
- total surveillance-depending on hospital guidelines
- targeted surveillance - e.g. to reduce catheter assoc infection
- outbreak surveillance

Recommended air Changes

- Clean areas - ventilation rate ≥ 20 ACH
- Prep rooms for sterile set-up ~37 ACH
- Ventilation - Air Distribution
  - Directional airflow from clean to dirty
  - Air introduced at ceiling and exhausted at floor
  - Downward movement of clean air
  - Airflow visualization (smoke testing)
  - Pressure differentials between areas
  - Need to keep doors closed

Laboratory ventilation system

CDC recommends -

- One pass non recirculating system
- Pass from clean to dirty area
- Highest pressure in corridor, negative pressure inside laboratory room
- Media preparation room should be under more positive pressure than general laboratory
- Isolation room should be under negative pressure with exhaust air moving through a HEPA filter
- 6-12 air changes/hour
- Aerosol generating procedures - in biosafety cabinets

THANK

YOU