Episodes of Single-Source, Multiple Laboratory Infections

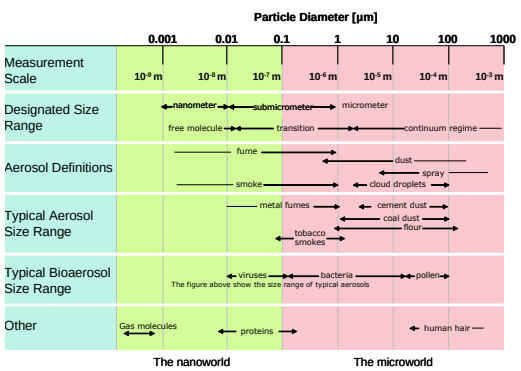
Disease	Probable Source of Infection	Maximum Distance From Source	Number Persons Infected
Brucellosis	Centrifugation	Basement to 3rd floor	94
Coccidioidomycosis (mammalian fungal disease)	Culture transfer solid media	2 Building floors	13
Coxsackie Virus infection	Spilled tube of infected mouse tissue on floor	5 feet (estimated)	2
Murine Typhus	Intranasal inoculation of mice	6 feet (estimated)	6
Tularemia	20 petri plates dropped	70 feet	5
Venezuelan encephalitis	9 lyophilized ampoules dropped	4th floor stairs to 3rd or 5th	24

Most slides from WHO/NIH

Reitman and Wedum, 1956

What is an Aerosol?

- Particles suspended in a gas
- For our purposes the gas is air

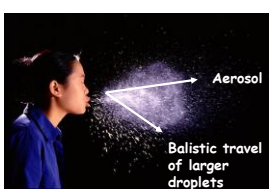


Approximate Size Ranges of Various Bioaerosols

Particles	Diameters (µ)
Smoke	0.001 – 0.1
Viruses	0.015 – 0.45
Bacteria	0.3 – 5
Cat Ag-bearing particles	<2.5 – 15 (most >5)
Fungal spores	2.0 – 50
Algae cells/clusters	1 – 100+
Protozoa	2 – 100+
Dermatophagoides fecal pellets	~20
Fern spores	20 – 60
Pollen	10 - 100

Droplet evaporation time and falling distance

Diameter of droplet (µm)	Evaporation time (s)	Distance fallen before evaporation (m)
200	5.2	7.2
100	1.3	0.45
50	0.31	0.03
25	0.08	0.002

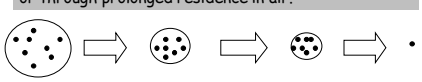


Characteristics of droplet infections

Due to their heavier weight larger droplets might be expected to deposit on mucous membranes of the upper respiratory tract

Nuclei and aerosols may penetrate into the pulmonary alveoli

Unlike MTB there is no evidence for influenza infection over long distances (e.g. ventilation systems) or through prolonged residence in air.



Dynamic process of Adsorption, Evaporation and Concentration

Formation of solid particles (nuclei) from droplets

Characteristics of droplets and aerosols

	Droplets	Aerosols/Nuclei
Diameter	>5µm	< 5µm
Generation	Coughing/Sneezing	Lab techniques
Evaporation of water shell	No	Yes/not nuclei
Sedimentation	Rapidly	Slowly
Transmission	On surfaces	Air

Aerosols Produced from Laboratory Operations

10^{10} bacteria/ml culture – 10 min

Blender, opened at once	10^6
Sonicator, with bubbling	10^6
Pipetting, vigorous	10^6
Dropping culture	3×10^5
Splash on centrifuge rotor	10^5
Drop spill on zonal rotor	2×10^4
Blender, opened at 1 minute	2×10^4
Pipetting, carefully	10^4

Dimmick et al., 1973

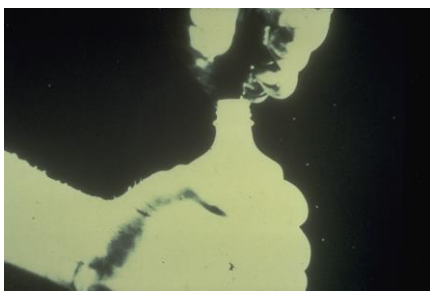
Aerosol and Surface Recovery from Pipetting Operations of 10^9/ml *B. subtilis*

RUN	Airborne CFU	Settled CFU	
		Hands	Area
1	2,040	35,800	3,700
2	657	22,000	860
3	2,050	14,800	1,700
4	388	9,300	550
5	5,110	6,900	2,100
6	649	228,000	2,900
Average	1,820	52,800	1,970

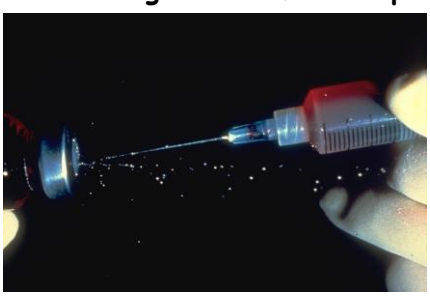
(average time 3 minutes; 1 ml pipette; ea. 2 ml bulb)

Chatigny et al, 1979

Unscrewing Bottle Cap



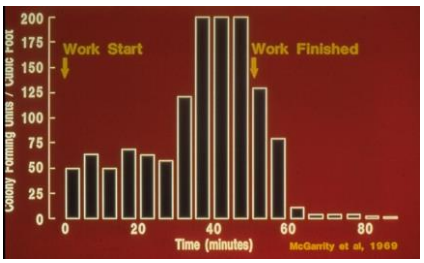
Withdrawing Needle from Septum



Vortex Mixing

Blowing Out the Last Drop

Aerosols from Animal Cage Cleaning



What determines risk from aerosols?

- size of particle (5 µm --> 0.5µm)
- concentration of pathogen
- Risk Group of agent (RG1-4)
- amount of aerosol produced by the procedure
- dilution of aerosol in air
- survival of agent

Factors affecting survival of agents in aerosols

- concentration worked with
- properties of the agent itself
- environment i.e. temperature, relative humidity, sunlight
- medium: pH, nutrients, organic material
- surface: porous / non-porous

Summary

- Aerosols are generated from most laboratory tasks
- Aerosols can spread through a building and effect many people
- Contamination is often heaviest in work areas and on worker hands

Minimizing Aerosols

- Wouldn't it be nice if we could see laboratory generated aerosols this well?
- It is often difficult to detect or measure laboratory aerosol production

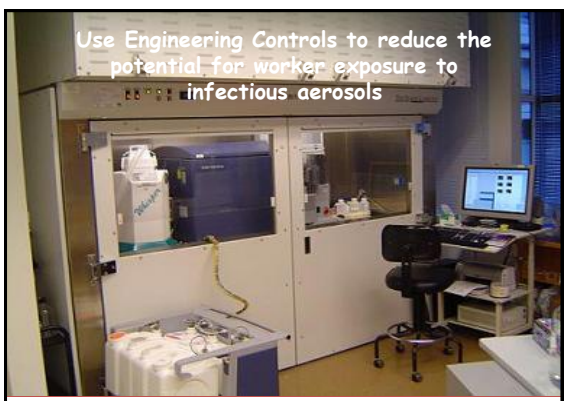
The Hierarchy of Controls

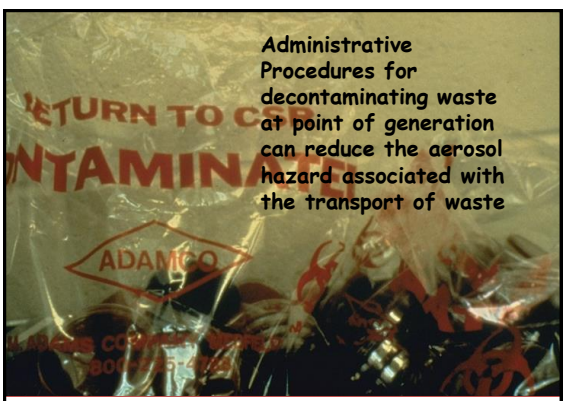
Whenever possible the hazard should be:

- Eliminated/Substituted
- Minimized through engineering controls
- Further controlled through administrative procedures
- Respiratory protection (PPE) should be used as a last resort for worker protection if hazard elimination, engineering controls, and administrative procedures do not sufficiently reduce worker exposure

Hierarchy of Controls with examples for Biological Agents

Control	Comment	Example
1) Elimination	Redesign the job or substitute a substance so that the hazard is removed or eliminated.	Treating a sample before handling to eliminate biological hazard.
2) Substitution	Replace the material or process with a less hazardous one. Care should be taken to ensure the alternative is safer than the original.	Replace virulent strains with attenuated ones e.g. use Sterne strain of B.anthracis rather than a clinical one or influenza PR8 rather than a current circulating H1N1 strain.
3) Engineering controls	Use work equipment to prevent exposure to infectious agents where they cannot be avoided. Install or use additional safety machinery. Separate the hazard from the operator by methods such as enclosing or guarding of machinery/equipment. Give priority to measures which protect collectively over individual measures.	Can the work be enclosed, vented, trapped or filtered? Use Class 1, 2, 3 biological safety cabinets or individually ventilated animal cages. Use appropriately constructed facilities etc
4) Administrative controls i.e. operational controls	These are all about identifying and implementing the procedures needed to work safely. Minimise quantities used. Minimise numbers of people potentially exposed	For example: good microbiological practice, techniques and procedures. Restricted access to hazardous areas; increasing safety signage, and performing risk assessments
5) Personal protective clothing and equipment	Only after all the previous measures have been tried and found ineffective in controlling risks to a reasonably practicable level, must personal protective equipment (PPE) be used.	PPE will reduce exposure of skin, eyes and potentially lungs. If chosen, PPE should be selected and fitted by the person who uses it. Workers must be trained in the function and limitation of each item of PPE.





Good Laboratory Practice Can Help Minimize Risks from Potentially Infectious Aerosols

Examples

- Use pipetting devices
- Eliminate or reduce use of open flames and heat sources
- Use disposable inoculating loops, if available
- Do Not dispose of used pipets in vertical discard containers
- Containerize infectious materials for transport

- Use additional exposure-reducing protective measures based on risk assessment
- Additional respiratory protection may be necessary in some Biosafety Level 3 laboratories
- One option is the Positive Air-purifying Respirator (PAPR)

What Types of Equipment are Available to Help Minimize Aerosol Production?

- Syringes, needles and accessories
- Blending devices
- Centrifugation tubes and accessories
- Heating and sterilization devices
- Autoclaves
- Collection systems
- Containers and flasks
- Pipetting aids and devices
- Miscellaneous equipment

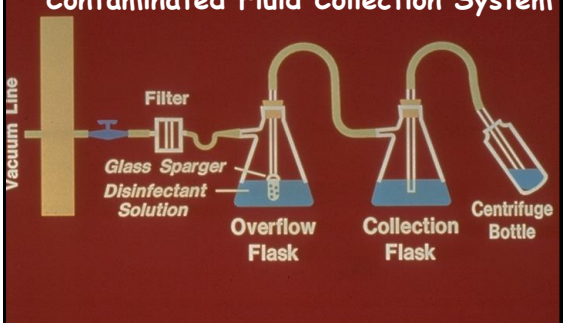
Sonicators, Homogenizers and Shakers Present Special Problems

Sealed or Capped Centrifuge Tubes

Sealed Centrifuge Bucket Assembly

- Ensure o-rings are intact
- Open only inside BSC
- Do not overfill tubes
- Check tubes for cracks

Contaminated Fluid Collection System



Best Practices for Pipetting Infectious Materials

No Vertical Discard of Pipettes

- Use only horizontal pipette discard pans
- Draw disinfectant in to the pipette and gently submerge

Safety Evaluation and Selection of Pipetting Aids

Choose Pipette Aids that:

- Will hold liquid without leakage
- Prevent contamination of vacuum lines
- Can be cleaned and disinfected
- Protects worker by eliminating mouth pipetting

Summary

- All personnel in infectious disease laboratories should be aware of the hazards associated with the creation of aerosols from laboratory operations and equipment.
- All manipulations of infectious materials generate aerosols to some degree.
- Personnel should be familiar with procedures for minimizing the generation of potentially infectious aerosols.
- Aerosol hazards should be eliminated where possible and engineering controls and administrative procedures should be implemented to further reduce risks.
- Use of respiratory protective devices may be warranted after risk assessment in conjunction with engineering and administrative controls.
- Splashes, sprays, drips and needlesticks also need to be controlled