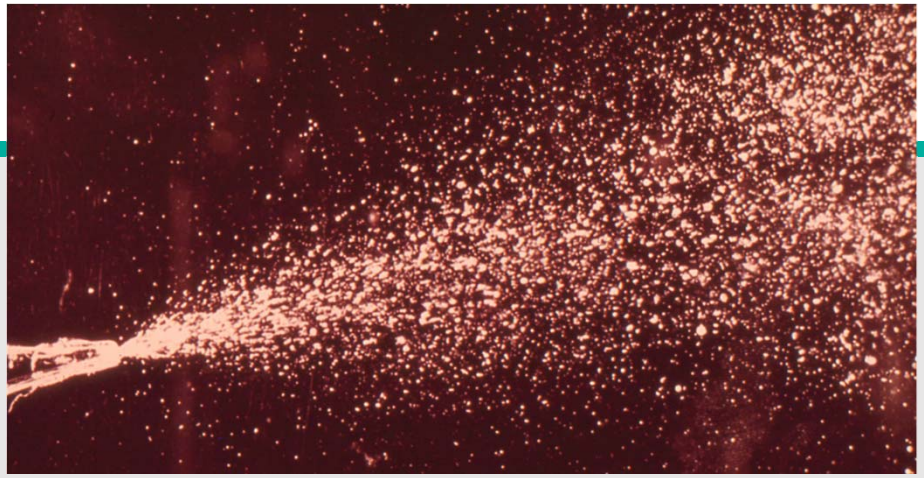


Microbial aerosols and the design of containment laboratories

Allan Bennett
Biosafety
UKHSA

The microbiology laboratory is designed to protect workers and the environment from aerosols

Aerosol containment equipment and techniques

- HEPA filters
- Safety Cabinets
- Directional airflow
- Negative pressure
- Isolators
- IVC

Where are the aerosols in the modern microbiology laboratory and how effective is this equipment in preventing laboratory infection?

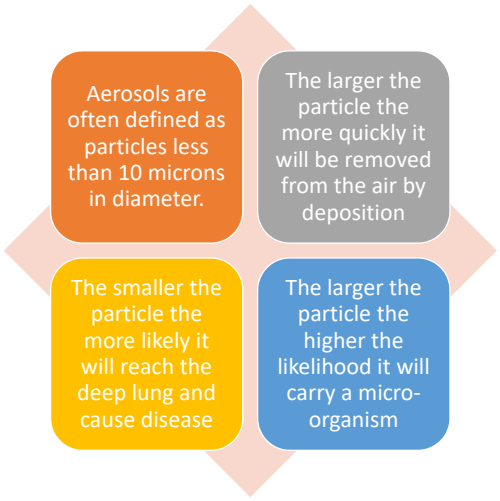
Introduction

- Microbial aerosols and disease transmission
- Microbial aerosols and laboratory infections through the years
- Where are microbial aerosols generated in the microbiology laboratory?
- How effective is laboratory equipment at containing microbial aerosols
- Risk assessment and microbial aerosol exposure

Aerosol Transmission

- Difficult to control
- Difficult to prove
- Therefore, the contribution of aerosol transmission to diseases can be controversial within and without the microbiology laboratory
- To understand aerosol transmission we need to understand microbial behaviour in the airborne state

Particle Size



Deposition - Stoke's Law

Deposition Velocity (u) = $\frac{\rho d_p^2 g}{18\mu}$

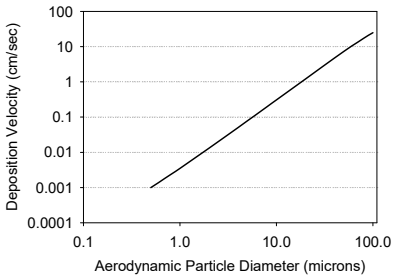
ρ- density of particle, μ- viscosity of air, g-gravity,

d_p- particle diameter

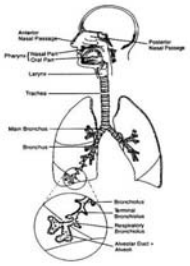
The deposition velocity is directly proportional to the particle diameter squared

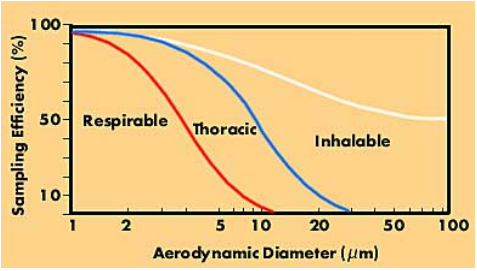
Particle Deposition

- Deposition Velocity (u) = $\rho d_p^2 g/18\mu$
- The deposition velocity is directly proportional to the particle diameter squared
- 2 micron particles will deposit at 0.72cm/min in still air
- 20 micron particles will deposit at 72cm/min in still air
- Evaporation



Particle Deposition in the Respiratory Tract

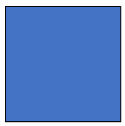




Effect of Particle Size (1)

1 micron diameter particle

2 micron diameter particle

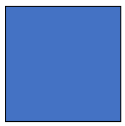


4 micron diameter particle

Effect of Particle Size (2)

1 micron diameter particle. Volume = 1x1x1 = 1 cubic micron

2 micron diameter particle. Volume = 2x2x2 = 8 cubic microns



4 micron diameter particle. Volume = 4x4x4 = 64 cubic microns

Effect of Particle Size (3)

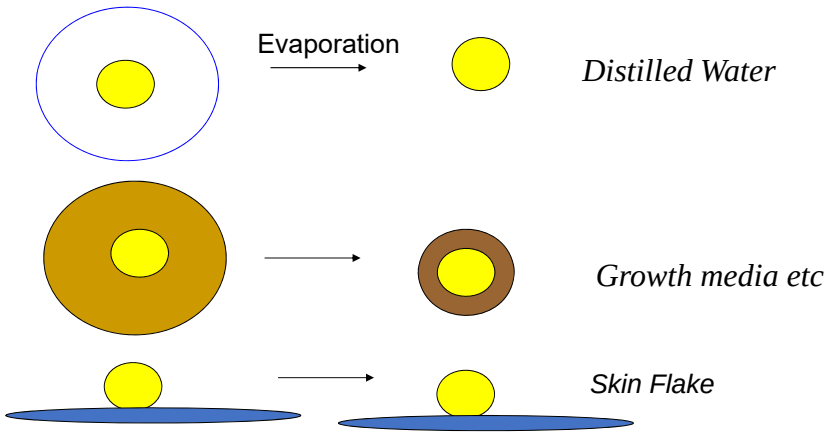
Concentration of spray suspension is 1 virus per cubic micron (10^{12} virus per ml)

1 micron particle. Vol = 1 cubic micron. 1 virus per particle

2 micron particle. Vol = 8 cubic microns. 8 virus per particle

4 micron particle. Vol = 64 cubic microns. 64 viruses per particle

Aerosol Particle Size is Not the Size of the Micro-organism



Aerosols in the Laboratory

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Microbial aerosols and the design of containment laboratories

Introduction

Aerosol infections in the laboratory

Aerosol sources in the laboratory

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Microbial Aerosol Formation

- Energy is required to form an aerosol particle from a liquid
- This is needed to break the surface tension of water
- In most instances only a small proportion of a liquid will be aerosolised

Laboratory Acquired Infections (1910-50) - Aerosols & Outbreaks

- A Sharples centrifuge used for centrifuging live Brucella organisms led to 45 clinical cases and one death. The centrifuge was located in the hallway of the basement.
- 20 lab workers infected with VEE when 9 freeze dried ampoules dropped
- Numerous Q fever outbreaks associated with buildings centrifuging and blending infected eggs
- 3 died of glanders following centrifuge accident
- 11 cases of typhus due to intranasal infection of mice
- Many of these associated with biological weapons research. High titre, high aerosol risk.

Making the Laboratory Safer (1950-1970)

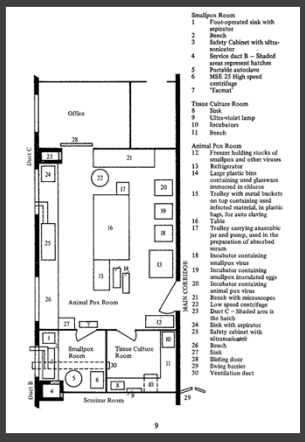
- Biosafety Pioneers – Fort Detrick, Porton Down, Geelong etc
- Developed Safety Cabinets
- Built the evidence base
- Limited in the main to large facilities handling large volumes and titres
- Standards developed
- First regulations

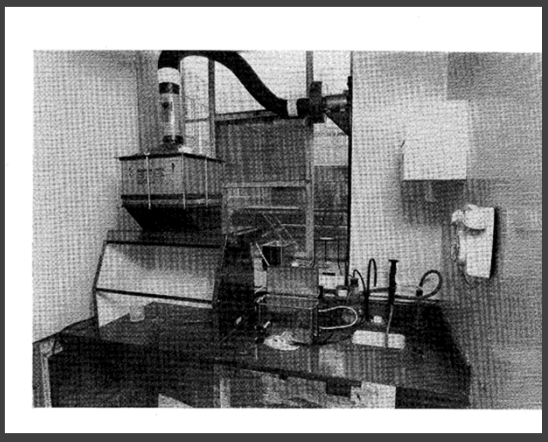
Laboratory Acquired Infections (Pike 1976)

CAUSE

Accident	17.9
Animal or ectoparasite	16.8
Clinical Specimen	7.3
Glassware	1.2
Autopsy	1.9
Intentional Infection	0.5
Aerosols (known)	13.3
Work with agent	21.1
Others, unknown	20.0

Brucellosis	426 (5)
Q fever	280 (1)
Hepatitis	268 (3)
Typhoid fever	256(20)
Tularemia	225 (2)
Tuberculosis	194 (4)
Typhus	124
Psittacosis	116 (10)
Leptospirosis	78
Streptococci	87



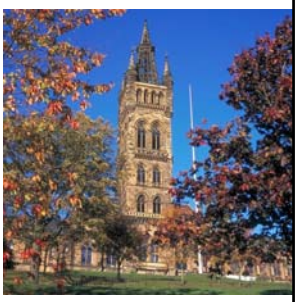


Last Case of Smallpox Birmingham England 1978

- Last reported case of smallpox. Photographer in medical department and mother
- Smallpox laboratory housed in facility funded by WHO. Inspected and passed for use by UK regulator though WHO expressed reservations about safety practices.Lab to be closed 1978
- Head of Department later committed suicide
- Public Enquiry accepted a theory that the infection was spread by an aerosol through a ventilation to the photographer's office
- However, there weremany other theories
- This incident led to an increase in the specifications for aerosol control in the microbiology laboratory which were written in national and international reulation

When the original WHO Biosafety Manual was first written, 1983, and I was a student

- No automatic pipettes
- Mouth pipetting common
- Few genetic techniques
- Most work carried out on the bench. Few safety cabinets
- Smoking in labs was common
- Laboratory infections
- TB incidence in lab technicians 59 per 100,000 (4 times normal incidence) Grist & Emslie
- *Strep pyogenes* handled in a 1st year undergraduate class – Strep throat



Where are microbial aerosols generated in the microbiology laboratory?

Original work by Dimmick and Kenny and Sabel published 1968 -1973

However techniques used are largely irrelevant to today' microbiology laboratory

Large scale accidental release

Failure	Aerosol Concentraion (spores per m^3)
Filter Failure	0
Antifoam failure	$2.4 \times 10^5 – 1.1 \times 10^7$
Pipework failure	$1.4\text{-}3.7 \times 10^6$
Glass vessel shatter	$1.3\text{-}1.5 \times 10^6$
Metal Rupture	$1.4\text{x } 10^6\text{- } 4.9 \times 10^7$

- Interested in aerosol generation from fermenters
- Ashcroft and Pomeroy mimicked fermenter failures using a variety of means including plastic explosives
- 10^{10} spores per ml suspension
- Ashcroft, J., & Pomeroy, N. P. (1983). The generation of aerosols by accidents which may occur during plant-scale production of micro-organisms. *Epidemiology & Infection*, *91*(1), 81-91.

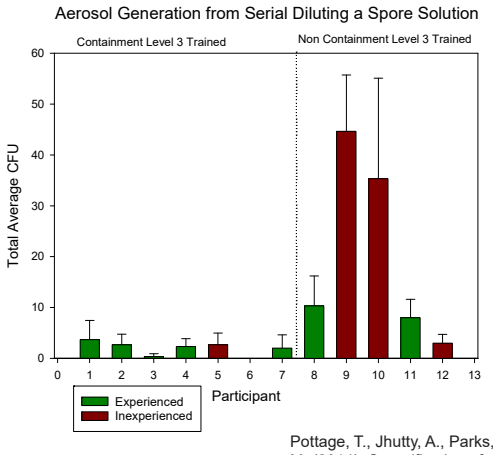
Aerosol Generation From Laboratory Accidents

Accident (10^9 spore/ml suspension)	Aerosol Generated (cfu/m^3)
Centrifuge Rotor Leak*	2.30×10^4
Flask Break in Shaking Incubator*	1.15×10^3
Dropping 250ml Flask	1.03×10^3
Dropping Large 2l Bottle*	1.37×10^4
15ml Spill from 1m*	2.07×10^3

* indicates > 50% of aerosol particles less than 3 microns diameter

Bennett, Allan, and S. Parks. "Microbial aerosol generation during laboratory accidents and subsequent risk assessment." Journal of applied microbiology 100.4 (2006): 658-663.

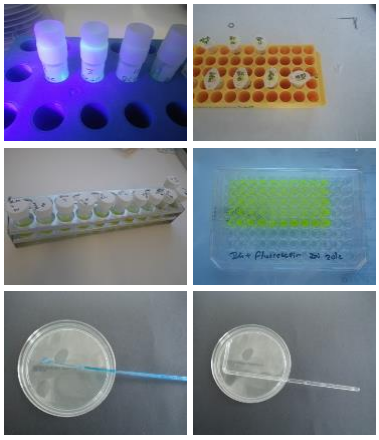
Aerosols from Serial Dilution – Effect of Training



Pottage, T., Jhutty, A., Parks, S. R., Walker, J. T., & Bennett, A. M. (2014). Quantification of microbial aerosol generation during standard laboratory procedures. *Applied Biosafety*, *19*(3), 124-13

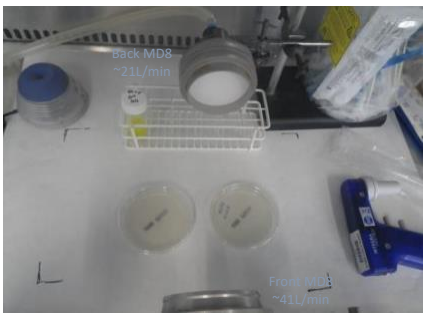
WHO funded study on aerosol generation in the modern microbiology laboratory - Methodology

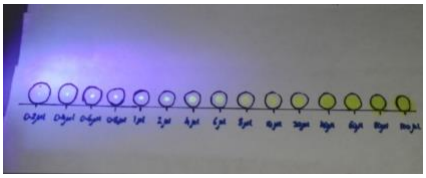
- Test suspension
  - *Bacillus atrophaeus* (BA) spores 10^9 cfu/ml with added sodium fluorescein (0.01%)
  - BA 10^7 cfu/ml with added fluorescein, for select procedures
  - Test procedure and sample volumes handled
    - Vortex mix and Hand shake mix
      - 1 ml – Cryo-tube and Eppendorf tube
      - 10 ml – Plastic universals
    - Pipette mix and Serial dilution
      - 0.1 ml – 96 well plate and Eppendorf tube
      - 1 ml – Cryo-tube and Eppendorf tube
      - 10 ml – Plastic universals
    - Plating out on solid media
      - 0.1 ml – Blue loop and Hockey stick
      - 0.2 ml – Blue loop and Hockey stick



25 Microbial aerosols and the design of containment laboratories

- Aerosol detection
  - two Sartorius MD8 sampler heads (front and back) close to the work area.
  - 5 min air samples (noted if longer)
  - 5 min cabinet vent between tests
  - Gelatine membrane filter incubated on TSA for 24hrs at 37°C.
  - Colonies enumerated and cfu/m^3 calculated
- Surface contamination
  - Absorbent white BenchKote
  - Observed under UV light

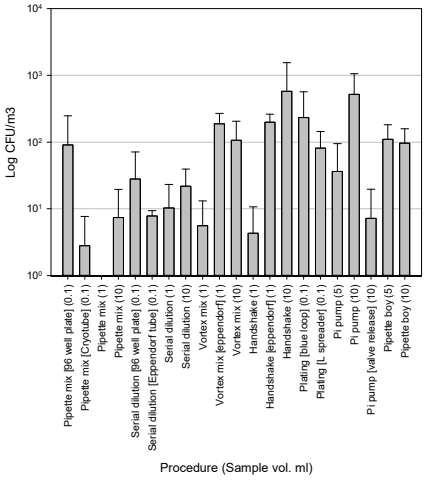




26 Microbial aerosols and the design of containment laboratories

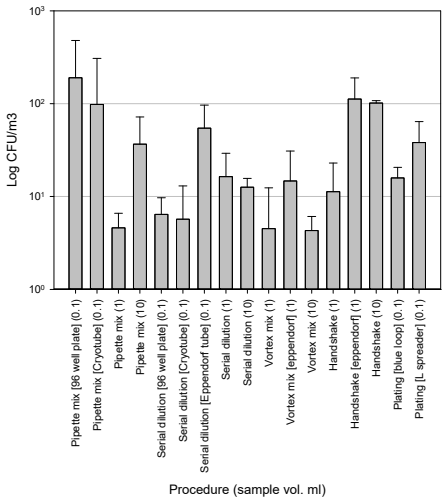
Aerosols Generated From Laboratory Procedures – High Titre

- Aerosol generated during procedures carried out by careless operator
- Pottage, T., Ngabo, D., Parks, S., Hookway, H., Verlander, N. Q., Kojima, K., & Bennett, A. M. (2022). Microbial Aerosols Generated from Standard Microbiological Laboratory Procedures. Applied Biosafety.



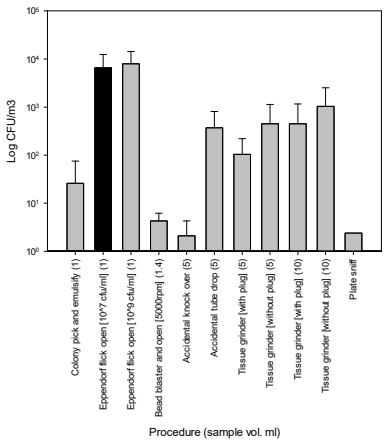
Aerosols Generated From Laboratory Procedures – Low Titre

- Generally lower titre meant lower aerosol concentration

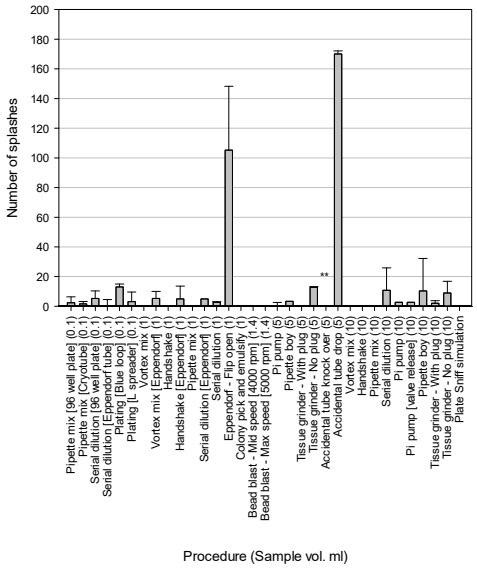


Aerosols Generated From Laboratory Procedures – Accidents

- Aerosols from accidents of higher concentration



Number of Droplets Generated From Laboratory Procedures –



Statistical Analysis – Relationship between aerosol formation and titre, volume and technique

Tests compared	Averages (SD)	Significance	Comment
Vortex mixing **10^9 cfu/ml** **1ml to 10ml**	1ml – 9(6) cfu/m³ 10ml – 134(79) cfu/m³	P=0.0047	Volume
Hand shaking **1ml volume** **10^9 to 10^7 cfu/ml**	10^9 – 755(406) cfu/m³ 10^7 – 97(26) cfu/m³	P=0.004	Titre
Pipette mixing **10^7 cfu/ml** **1ml to 10ml**	1ml – 5(1) cfu/m³ 10ml – 45(28) cfu/m³	P=0.026	Volume
Pi-Pump **5ml to 10ml**	5ml – 38(36) cfu/m³ 10ml – 522(289) cfu/m³	P=0.009	Volume
Pi-pump **Depress to valve use**	Depress – 522(289) cfu/m³ Valve – 23(12) cfu/m³	P=0.009	Technique
Eppendorf tubes **Vortex mixing then opening** **10^9 to 10^7**	10^9 – 189(67) cfu/m³ 10^7 – 15(13) cfu/m³	P=0.009	Titre

Microbial aerosols and the design of containment laboratories

How effective is laboratory equipment at containing microbial aerosols

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Protection factors

- A range of equipment is available in the laboratory to reduce operator exposure to microbial aerosols such as safety cabinets and respiratory protective equipment.
- They have assigned protection factors which are calculated as below

$$\text{Protection Factor} = \frac{\text{Aerosol concentration without protection}}{\text{Aerosol Concentration with protection}}$$

Measurement of protection factors

- In Europe potassium iodide aerosols are widely used to test safety cabinets in situ.
- Type testing of cabinets done with microbial aerosols normally spores
- Filters are tested with dispersed oil particles or other aerosol particulate but have been tested with bacteriophage
- Other methods can be used

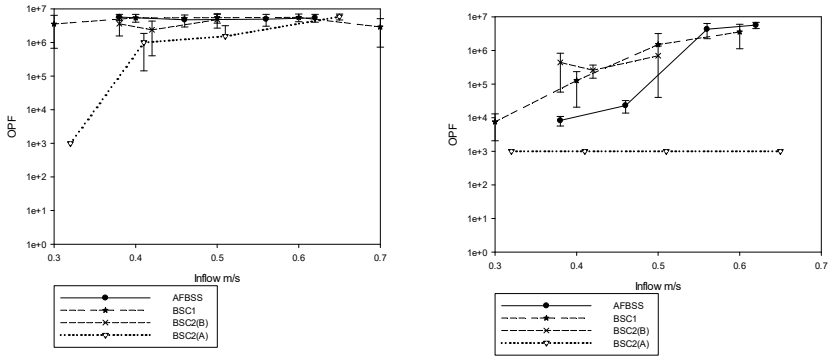
Protection Factors Against Aerosol Exposure

Device	Protection Factor
Nothing	1
Dust Mask	10
N95	20
HEPA filter 99.99%	10^5
Safety cabinet (1 or 2)	10^5 from in house data and KI testing
Class III safety cabinet and isolators	> 10^7 in house data

WHO study of sub-optimal Safety Cabinets

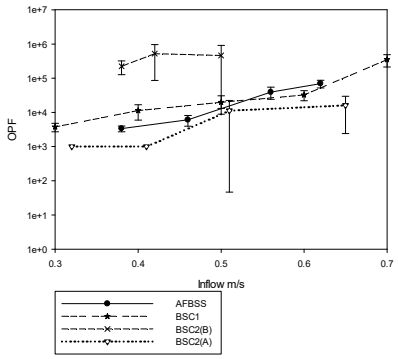
- Safety cabinets are mostly tested under optimal conditions
- This study used the standard KI test to measure the effectiveness of two MSC2 , 1 MSC1 and a CDC designed acid fast bacteria staining station (AFBSS) . Simply an aerosol is generated in the cabinet and sampled outside the cabonet
- The inflow velocity into the cabinets was varies and the cabinets were tested under the following conditions
  - A person walking in front of the cabinet
  - A door being opened next to the cabinet
  - A fan providing a cross draft
- The cabinet performance is specified as a operator protection factor
- Parks, Simon, Helen Hookway, Kazunobu Kojima, and Allan Bennett. "The Impact of Air Inflow and Interfering Factors on the Performance of Microbiological Safety Cabinets." *Applied Biosafety* (2021).

Standard Cabinet Testing v Walk By Testing



- BSC2(B) gave the best performance over all
- However, BSC2 (A) gave the worst performance even under normal operating conditions
- The BSC1 and AFBSS maintained a high level performance even under sub optimal conditions giving an OPF of greater than 4×10^5

Door Opening



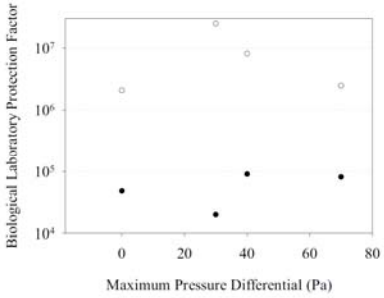
Microbial aerosols and the design of containment laboratories

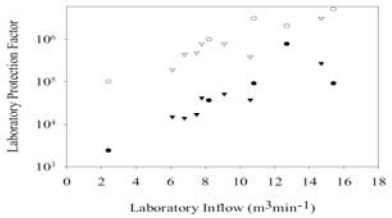
How much negative pressure do I need?

- A study was carried out using a particle tracer to assess aerosol containment in negative pressure laboratory and how it related to negative pressure and the provision of ante-rooms.
- An aerosol generator was placed close to the laboratory doors. Air was sampled in the anteroom and corridor as a person exited the laboratory.
- Bennett, Allan M., Simon R. Parks, and John E. Benbough. "Development of particle tracer techniques to measure the effectiveness of high containment laboratories." *Applied Biosafety* 10.3 (2005): 139-150.

Pressure Differential and Laboratory Inflow

- The containment of aerosols in laboratories was related to volumetric inflow and not to negative pressure
- Anterooms increased aerosol containment by up to 100 fold.





Theoretical Aerosol Dilution Times in Minutes

Air Change per hour	90%	99%	99.9%	99.99%
6	23	46	69	115
12	12	23	35	58
20	7	14	21	28
30	5	9	14	23
40	3	7	10	13

Assumptions

- The room air is well mixed
- No losses through deposition
- These probably cancel each other out

Risk Assessment

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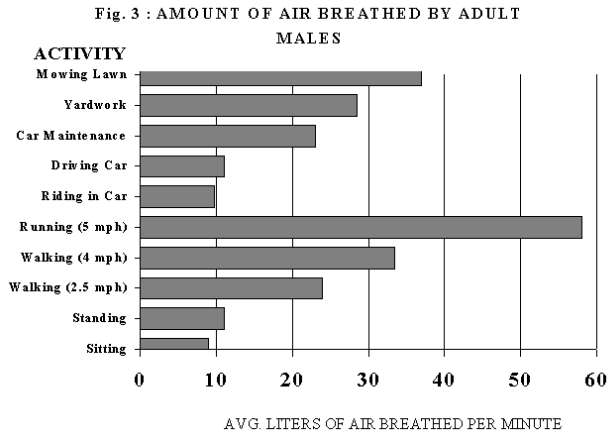
How can we use this information to measure risk to lab workers and the external environment

Calculating aerosol exposure

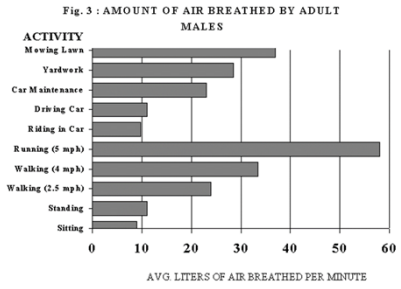
- The aerosol exposure of an individual is a function of concentration, duration of exposure and breathing rate
- Aerosol exposure (cfu) = Concentration ($cfum^{-3}$) x Exposure Time (min) x Breathing rate (m^3min^{-1})
- When containment equipment or PPE is used then we can incorporate the protection factor
- Aerosol Exposure = (C x Et x Br)/OPF

Breathing Rates



http://www.arb.ca.gov/research/resnotes/notes/94-11.htm

For normal purposes



- A cubic metre breathed per hour equates to light work.
- 16.7l/min is between driving a car and maintaining a car
- Therefore,

Aerosol exposure (cfu) = Concentration (cfum-3) x Exposure Time (min) x 0.0167

Risk Assessment of Various Laboratory Procedures

Procedure	Aerosol concentration (cfu/m³)	Exposure time (minutes)	Dose cfu - procedure on an open bench	Dose cfu – procedure within a BSC
Handshake (10ml) 10^9	580	10	87	0.00087
Pipette mix 10^7 (96 well plate)	190	10	29	0.00029
0.1ml serial dilution 10^7 (Eppendorf)	55	30	25	0.00025
Eppendorf flip off	8000	5	600	0.006

Releases from laboratory accidents

Procedure	Concentration (cfu/m³)	With HEPA extract (99.97%)
Centrifuge Rotor Leak*	2.30×10^4	0.69
Flask Break in Shaking Incubator	1.15×10^3	0.0345
15ml Spill from 1m*	2.07×10^3	0.0621
Dropping Large 2l Bottle*	1.37×10^4	0.411
Exploding Fermenter	1.5×10^5	4.5

Infectious Dose Data Needed for Full Risk Assessment

Disease	Transmission Route	ID50
Q Fever	Inhalation	10
Tuleraemia	Inhalation	10
Tuberculosis	Inhalation	1
Measles	Inhalation	1
Anthrax	Inhalation	>8000?
SARS-CoV-2		?????

Conclusions

- Microbiology Laboratories have been designed to contain microbial aerosols by the use of containment equipment, RPE and negative pressure
- Microbial aerosol generation occurs during laboratory procedures but the highest concentration detected are normally due to accidents
- Reducing titres and volumes handled will reduce aerosol risks
- There are many techniques available to measure the effectiveness of containment systems
- The potential exposure of laboratory workers to microbial aerosols can be easily calculated
- The current design of containment laboratories is very likely to protect against aerosol exposure of laboratory workers and release of microbial aerosols

Acknowledgements

- The microbial aerosol study and cabinet performance study was funded by World Health Organization in partnership with the Global Partnership Program of Global Affairs Canada
- The laboratory protection factor study was funded by Health and Safety Executive
- Simon Parks, Tom Pottage, Didier Ngabo, Anjeet Jhutty, Helen Hookway, etc