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Microbial Aerosols in the Modern Microbiological Laboratory

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Introduction

- Introduction to Microbial Aerosols
- Microbial Aerosols in the Laboratory
- Results
- Risk Assessment
- Microbial Aerosols Outside the Microbiology Laboratory
- Conclusions

Microbial Aerosols and Infection

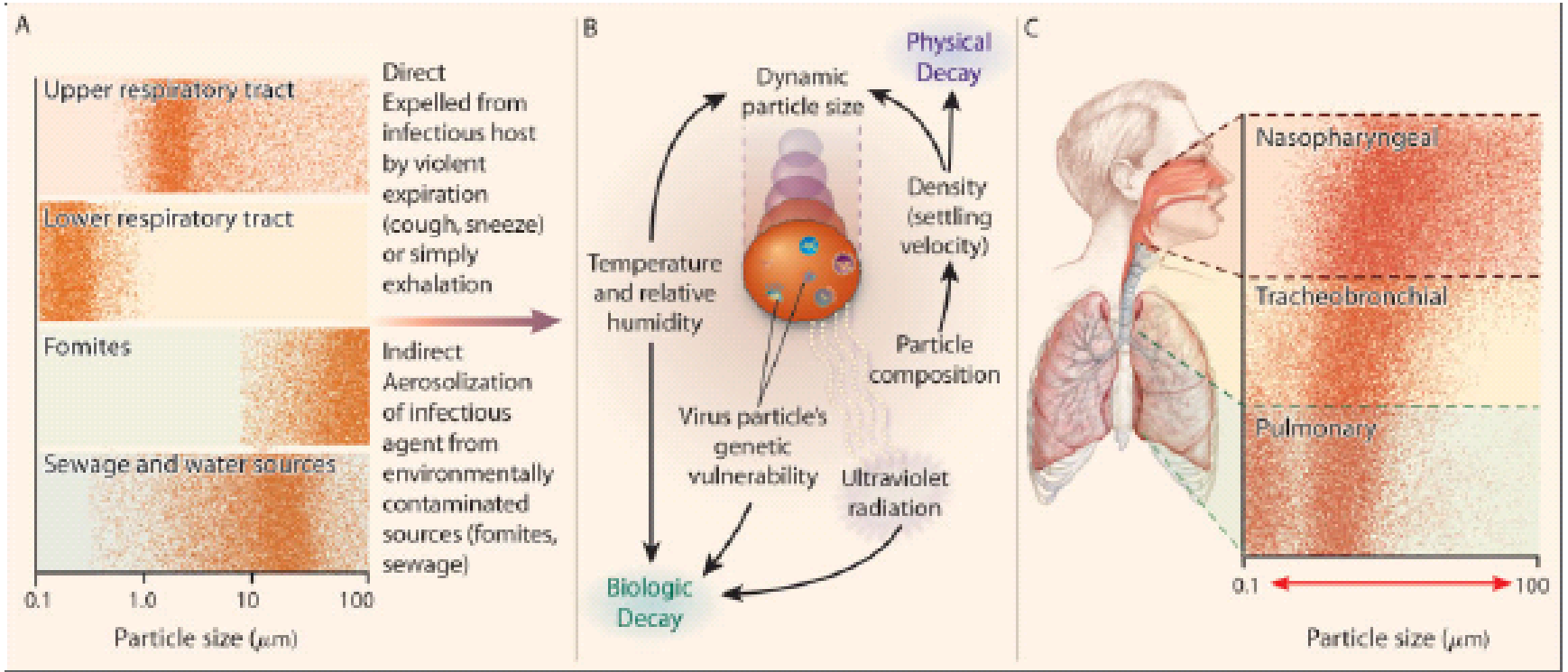

Figure. The Aerobiologic Pathway for the Transmission of Communicable Respiratory Disease.

Whether it is an infected human or a contaminated environmental matrix, each source (Panel A) generates particles with a characteristic range of sizes. The length of time a particle resides in the air (physical decay, Panel B) depends on its initial size, its composition, and environmental factors. Similarly, the length of time an airborne organism remains infectious (biologic decay) is affected by the infectious agent's initial metabolic state, genetic characteristics, and environment. The portion of the respiratory tract of a susceptible host in which inhaled particles are deposited (Panel C) is a function of the particles' aerodynamic size; in the middle of the range, particles may be deposited in both the upper and the lower airways.

Roy and Milton
NEJM 2004)

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Microbial Aerosols in the Laboratory

- Biosafety professionals in the 1960s and 1970s recognised the occurrence of Laboratory Associated Infections caused by microbial aerosols
- Researchers measured the aerosol generated from laboratory procedures
- To prevent exposure to microbial aerosols protective equipment was developed
  - Safety cabinets
  - Personal protective equipment
  - Negative pressure
  - HEPA filters
- All designed to prevent aerosol exposure of workers and the environment

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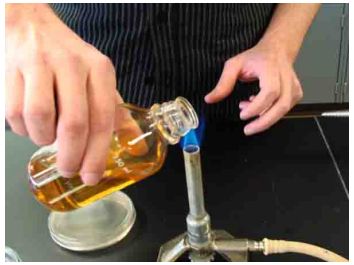
The microbiology laboratory has changed



1985

- Culture predominated
- Biochemical tests
- Uncontained centrifuges and homogenisers
- Large volumes (10ml test tubes)
- Pipette Aids

2019

- PCR commonly used
- MALDI-ToF, Sequencing
- Sealed rotors and other equipment
- Smaller volumes (Microtubes)
- Automatic pipettes and robotics

Has the risk of aerosol generation changed?

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Study Objectives

- To quantify microbial aerosol generation during common laboratory procedures
- To determine impact of following variables on aerosol quantity
  - Titre
  - Volume
  - Training
- To quantify surface contamination during common laboratory procedures
- To use data obtained in a risk assessment of operator exposure in the modern microbiology laboratory

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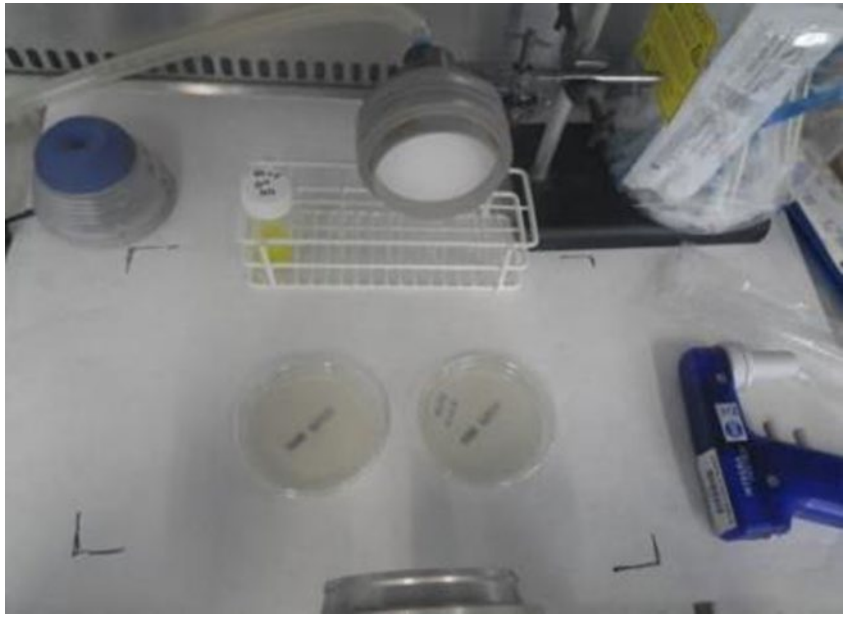
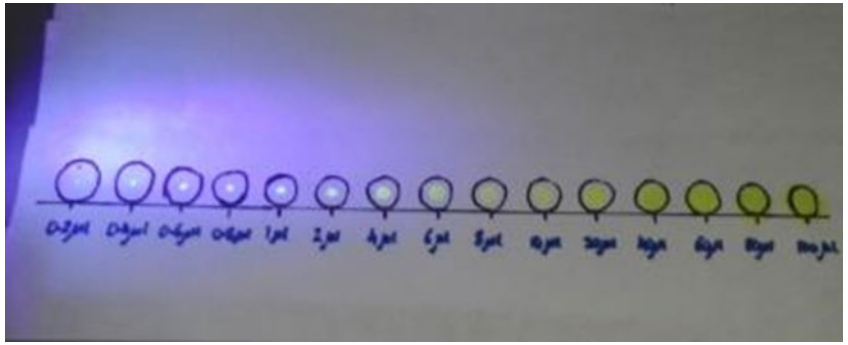
Study Methodology

- **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
- **Aerosol detection**
  - two Sartorius MD8 sampler heads (front and back) close to the work area.
  - 5 min air samples
  - 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





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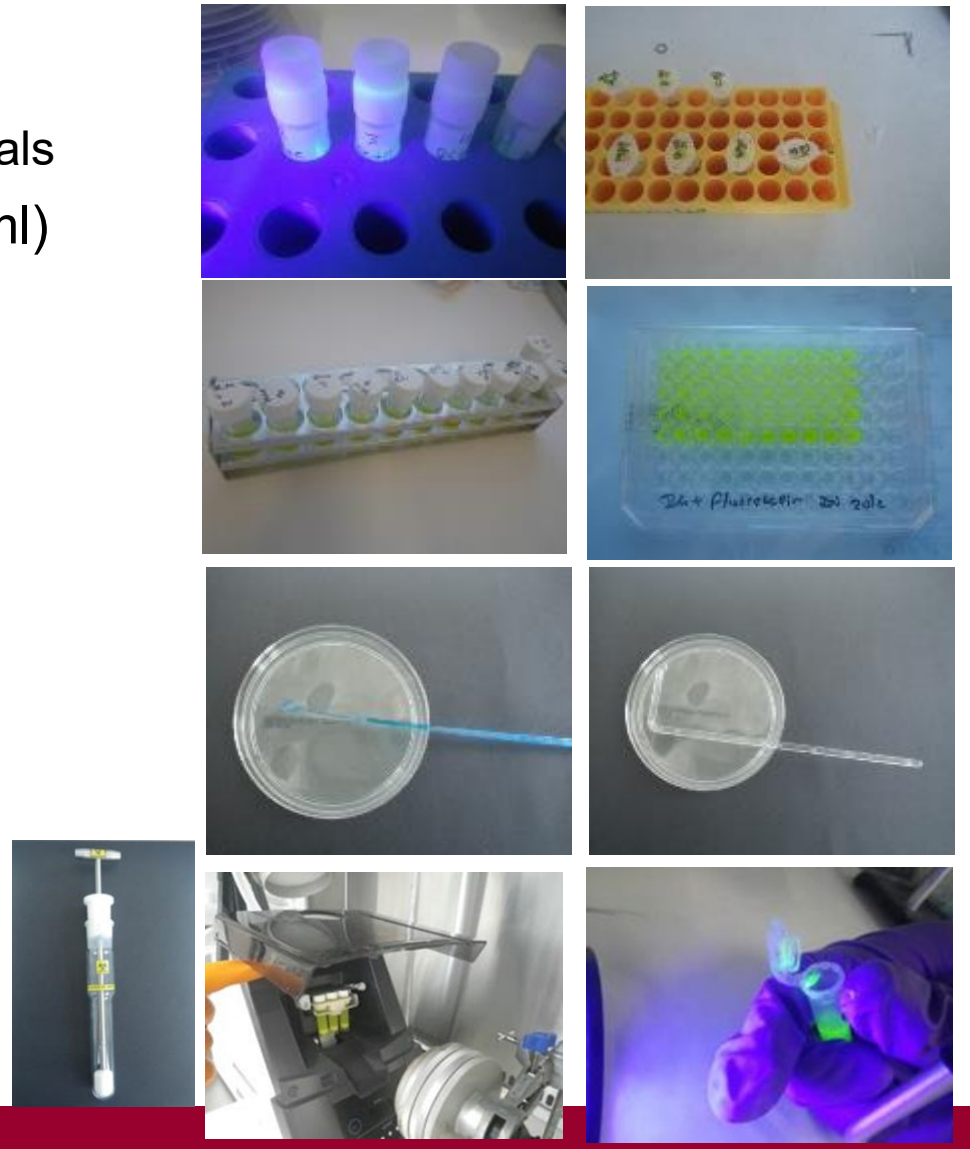
Procedures Studied

- Vortex mix and Hand shake mix (1ml,10ml)
  - Cryo-tube and Eppendorf tube, Plastic universals
- Pipette mix and Serial dilution (0.1ml, 1ml,10ml)
  - 96 well plate, as above
- Plating out on solid media (0.1ml, 0.2ml)
  - Blue loop and Hockey stick
- Sample aliquot
  - 5 ml – Pi-Pump and PipetBoy
  - 10 ml – Pi-Pump and PipetBoy
- Flipping open Eppendorf
- Plate sniffing simulation
- Tissue homogenisation
  - Bead blast
  - Tissue grinder



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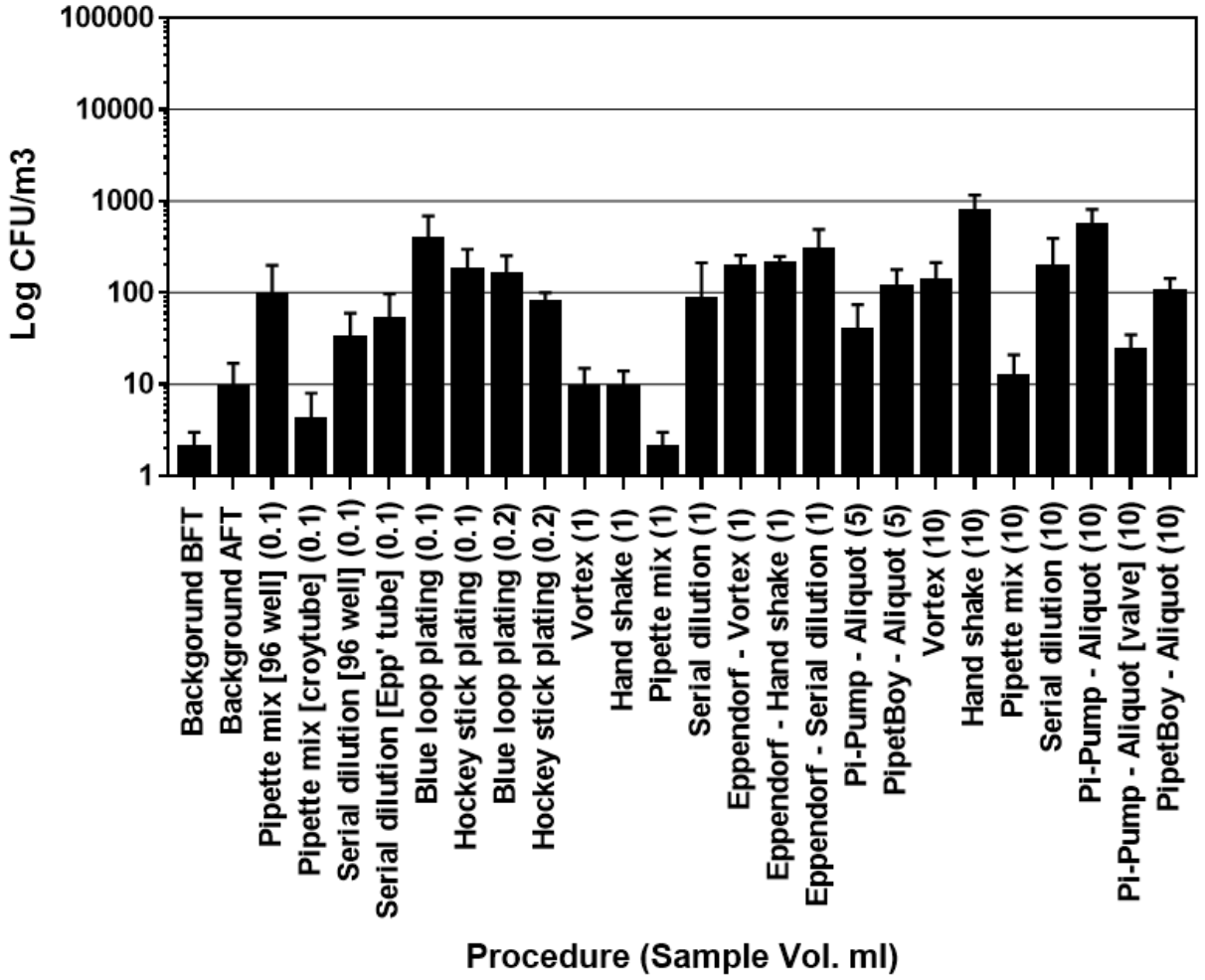
Aerosol Generation (10^9 cfu/ml)



- Aerosol concentrations from 4 to 950 cfu/m^3
- Some evidence of increases in aerosol generated with volume

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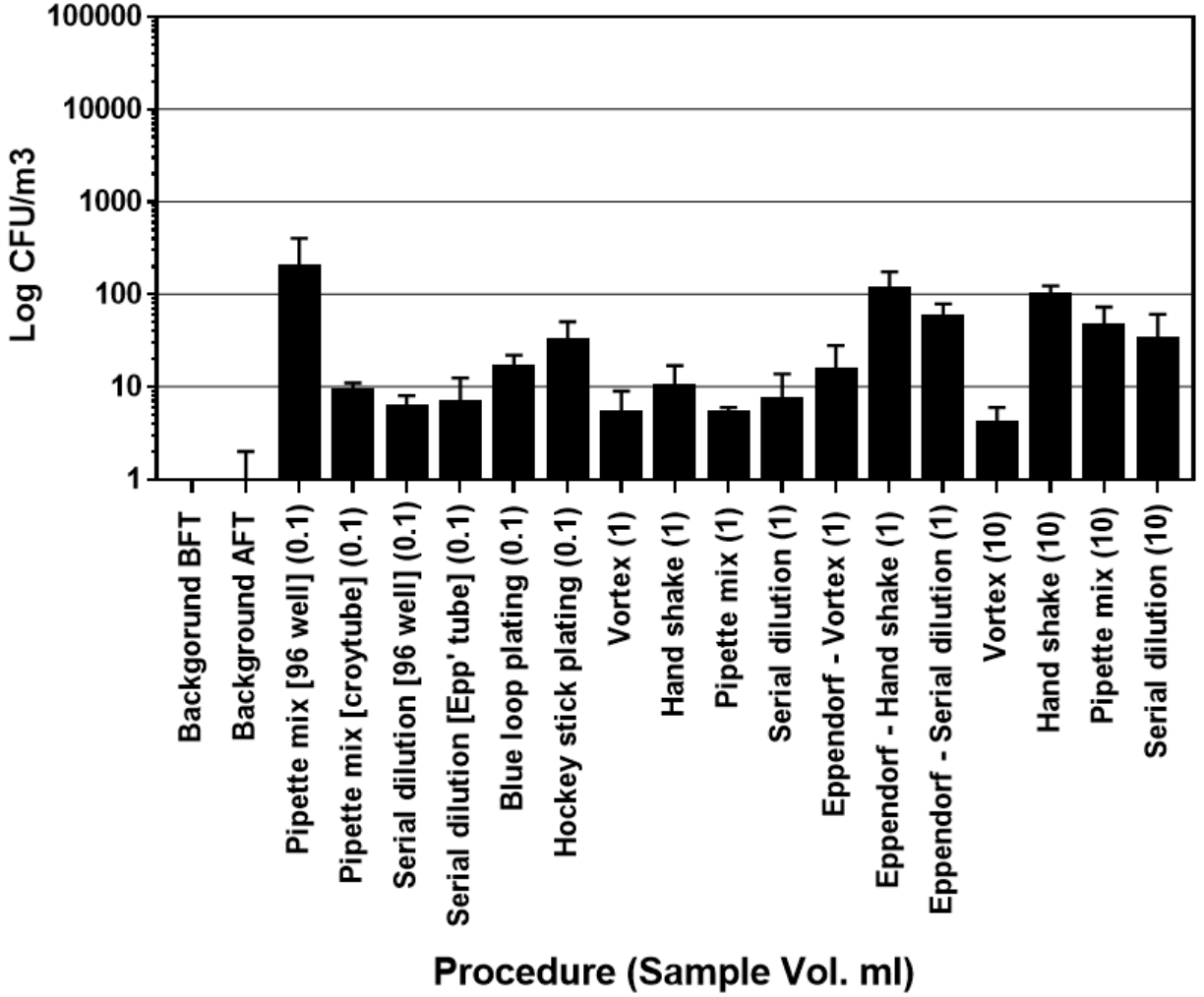
Aerosol Generation (10^7 cfu/ml)



- Aerosol concentrations from 5 to 200 cfu/m^3
- Evidence of reduction in aerosol generated with titre and perhaps volume

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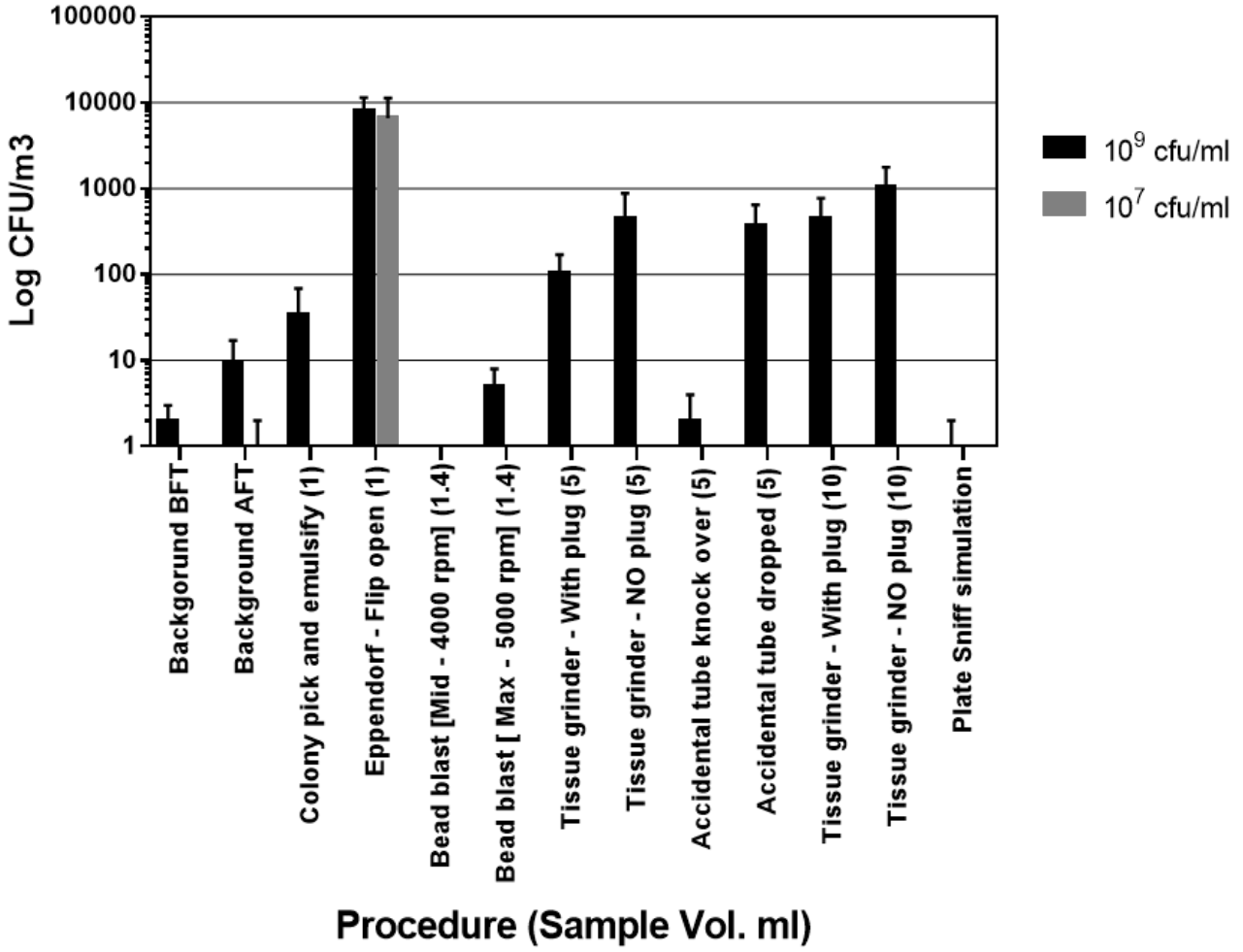
Other Procedures



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Statistical Analysis of Results

Tests compared	Average cfu/m^3	Significance	Comment
Vortex mixing 1ml to 10ml	1ml – 9 10ml – 134	P=0.0047	Volume
Hand shaking 10^9 to 10^7 cfu/ml	10^9 – 755 10^7 – 97	P=0.004	Titre
Pipette mixing 1ml to 10ml	1ml – 5 10ml – 45	P=0.026	Volume
Pi-Pump 5ml to 10ml	5ml – 38 10ml – 522	P=0.009	Volume
Pi-pump Depress to valve use	Depress – 522 Valve – 23	P=0.009	Technique
Eppendorf tubes Vortex mixing 10^9 to 10^7	10^9 – 189 10^7 – 15(13)	P=0.009	Titre

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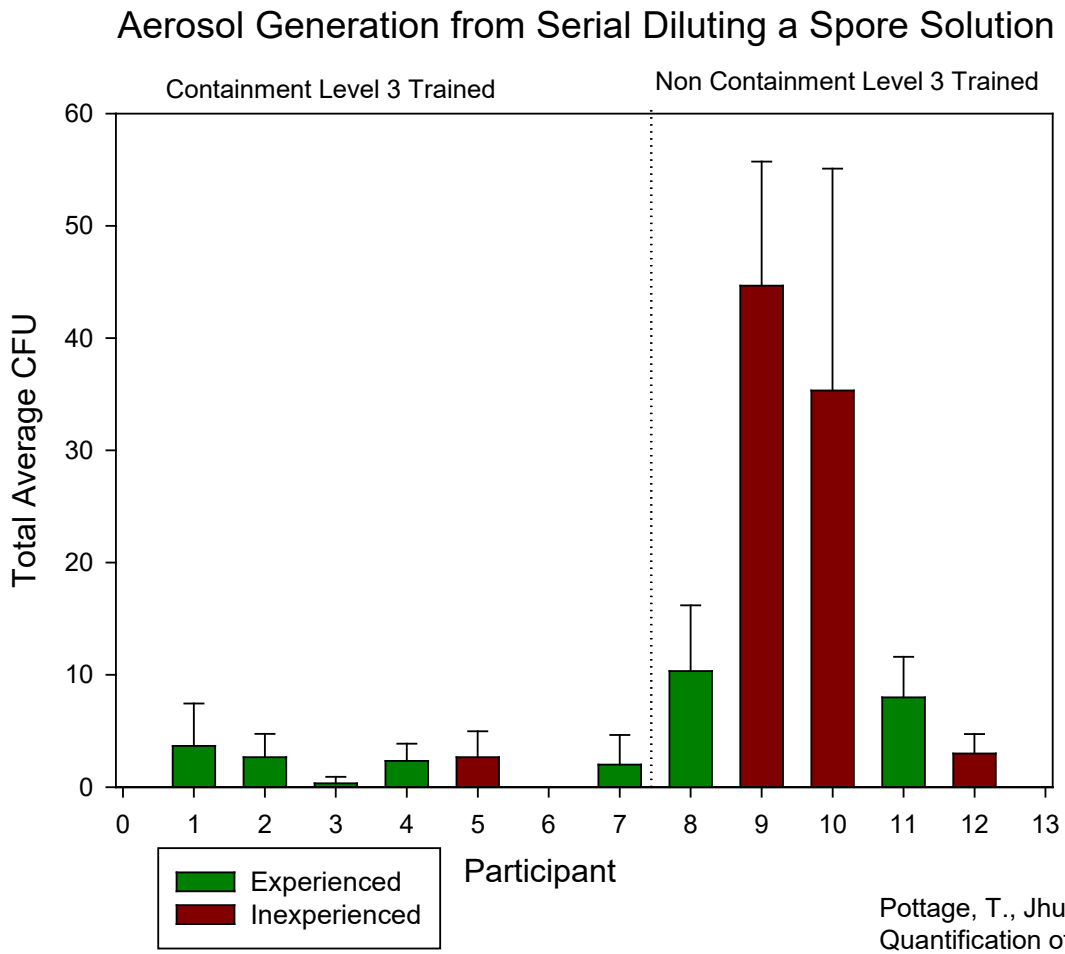
Training and Microbial Aerosol Generation



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), 124-131.

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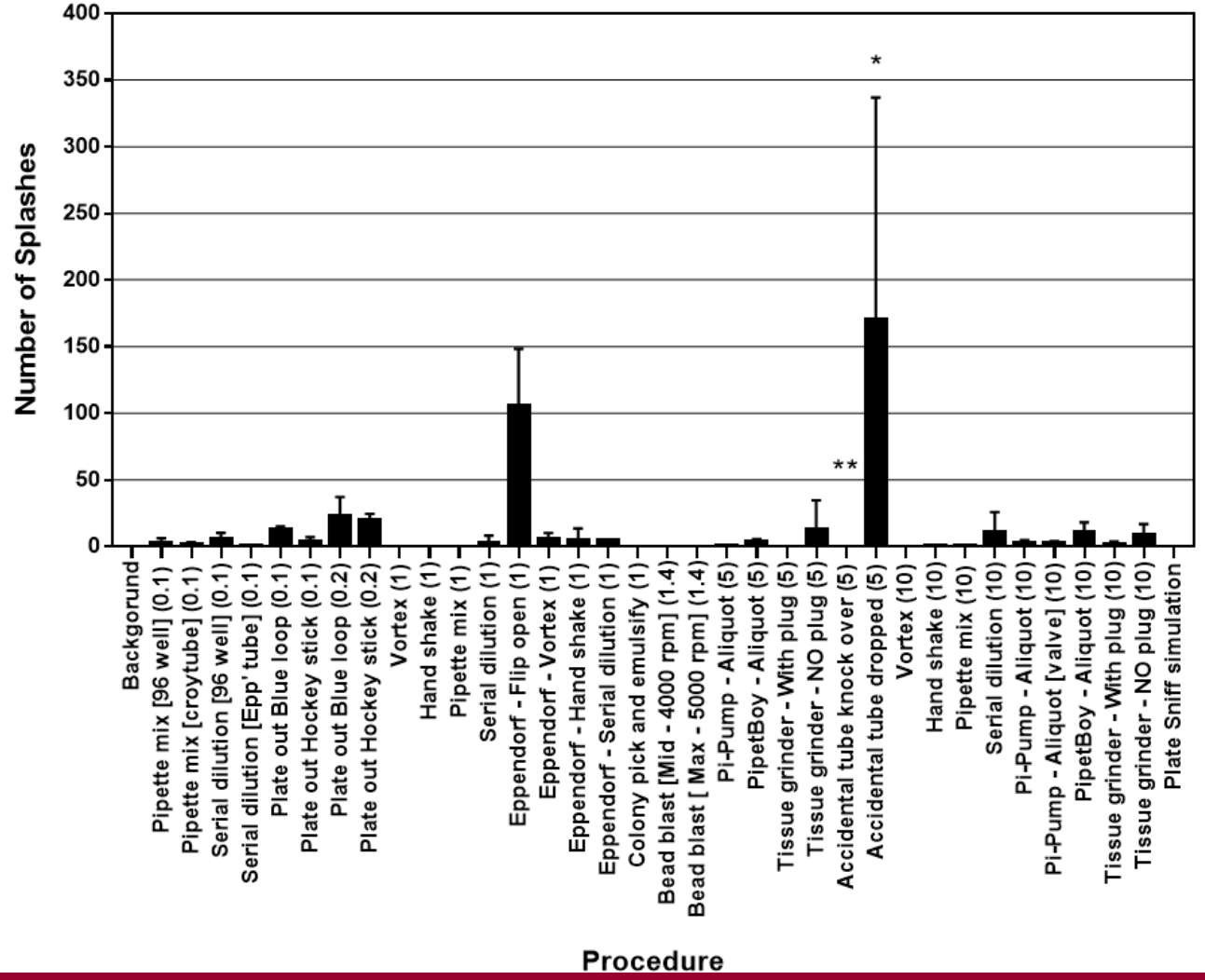
Surface Contamination



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When you flip an Eppendorf Lid

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Discussion

- Reducing volume and titre can decrease potential aerosol exposure
- Training also reduces levels of aerosol generated
- Certain procedures can generate significant aerosol but most commonly used processes can be undertaken with minimal aerosol generation
- This data can generally be taken as worst case for the following reason
  - Sampling was undertaken next to the process and represents aerosol generation not personal exposure
  - Samplers would have picked up large droplets which would probably not reach the worker and be inhaled by the worker
  - The work was not intended to be carried out to the highest levels of GMP
  - Many agents are less aerostable than *B. atrophaeus*

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Risk Assessment

- How can this data be used to measure potential operator exposure in the microbiological laboratory?

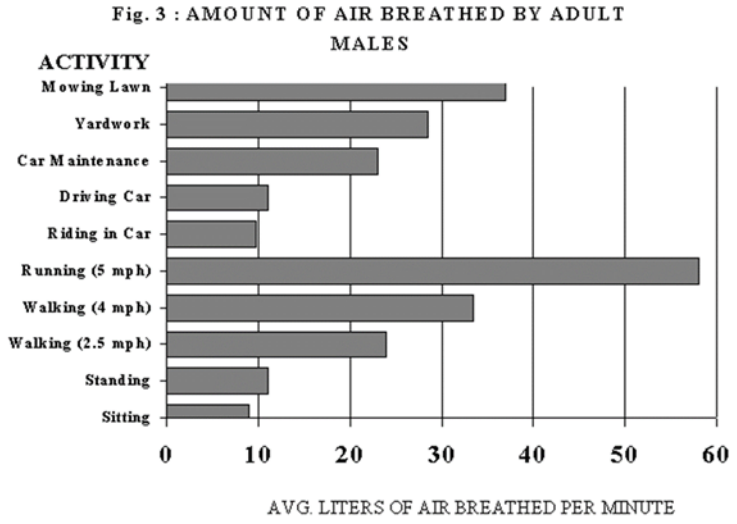
Operator exposure (cfu) = AC (cfu/m^3) x BR (m^3/min) x t (min)

Where

AC= Aerosol Concentration

BR = Breathing Rate (0.0167m^3/min)

T = exposure time



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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

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

The protection factor for a correctly operating safety cabinet is 10^5

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Serial Dilution (high concentration)

Volume	Aerosol Conc'n (cfu/m^3)	Containment	Potential Operator Dose per 5 minutes (cfu)
10ml	173	No Cabinet	14.4
		Correctly operating cabinet	0.00014
1ml	283	No Cabinet	23.5
		Correctly operating cabinet	0.00024
0.1ml	50	No Cabinet	4.2
		Correctly operating cabinet	0.000042

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Serial Dilution (10^7/ml)

Volume	Aerosol Con'nc (cfu/m^3)	Containment	Potential Operator Dose per 5 minutes (cfu)
10ml	32	No Cabinet	2.67
		Correctly operating cabinet	0.0000267
1ml	55	No Cabinet	4.5
		Correctly operating cabinet	0.000045
0.1ml	7	No Cabinet	0.58
		Correctly operating cabinet	0.0000058

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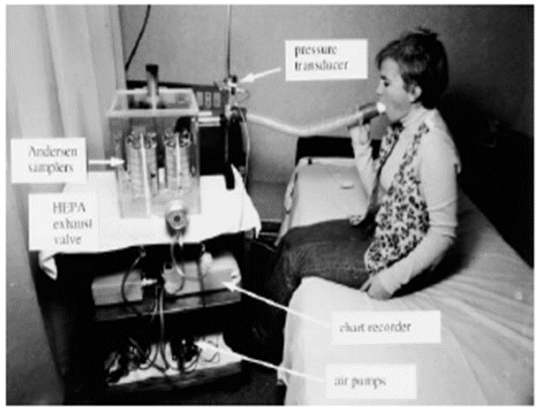
In comparison, microbial aerosols concentrations in healthcare are far higher

	Aerosol concentration per m^3
Tuberculosis patients (Fennely)	Up to 600 cfu
Influenza patients (Bischoff)	Up to 2 million RNA copies
Influenza patients in ICU (Thompson)	Up to 30 million RNA copies
Influenza non hospitalised students (Milton)	Mean 4.8 x 10^4 RNA copies in 30 mins
NTM in showers (unpublished)	4000 cfu



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Summary

- No process studied would have generated levels of microbial aerosol which would not be contained within a correctly functioning safety cabinet.
- Therefore none of the processes would need to be carried out within a negative pressure laboratory as an additional precaution against aerosol release or any additional respiratory protection required for operators
- Whether a cabinet is required to protect operators from aerosol infection will depend on the potential infectious dose by aerosol of the agent handled
- Low volumes, low titres and training in GMP further reduce the potential for aerosol exposure
- Aerosol levels during normal laboratory processes are lower than those found in other healthcare premises

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