Aerosols and Aerobiology

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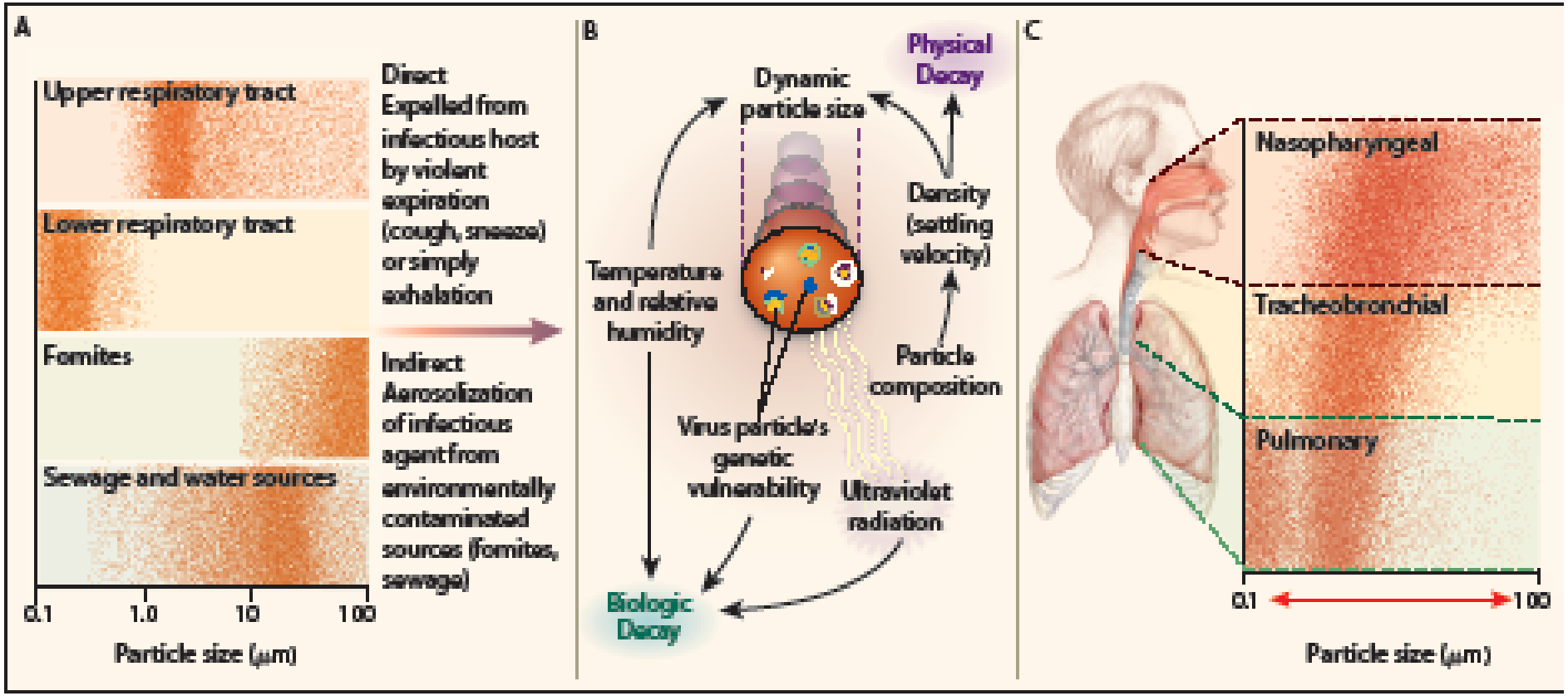
Aerosols and Aerosol-Acquired Disease

- Natural epidemics and airborne communicable disease
  - Few ‘obligate’ airborne pathogens
  - Nearly impossible to study dynamic phenomena empirically
- Experimental characterization & infection
  - Synthetic aerosols from anthropometrically-derived sources
  - Optimized for delivery, deposition

The aerobiologic pathway of communicable infectious disease



from Roy and Milton, NEJM, 2005

natural (communicable) and experimental infection

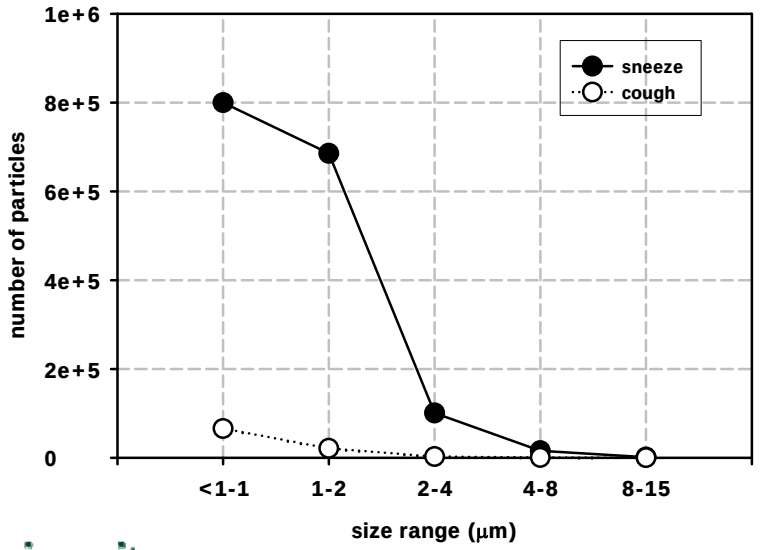
- ‘natural’ infection
  - heterogeneous
  - size/dispersion
  - (temporal) exposures
  - microbial characteristics
- experimental infection
  - homogeneity
  - synchronization

- aerosol-acquired disease
  - primary v. communicable (natural) infection
- **disease (model) development**
  - microbial susceptibility/infectivity
  - ‘quantal’ biological response
  - comparative pathogenesis/size modality

An exemplar of natural airborne infection: communicable transmission of M.tb

- transmission of *M.tb* in the context of aerosol exposure
- only obligate pathogen transmitted as in air/by aerosol
- models to study this phenomena
- corollary to vaccine & pathogenesis studies
- experimental infection uses the same size distribution (1-2 μm MMAD) regardless of model species

- modulation of particle size changes aerosol microbial efficiency
- What can be derived from the study of natural aerosol transmission of *M.tb*?



estimating the quanta of infection

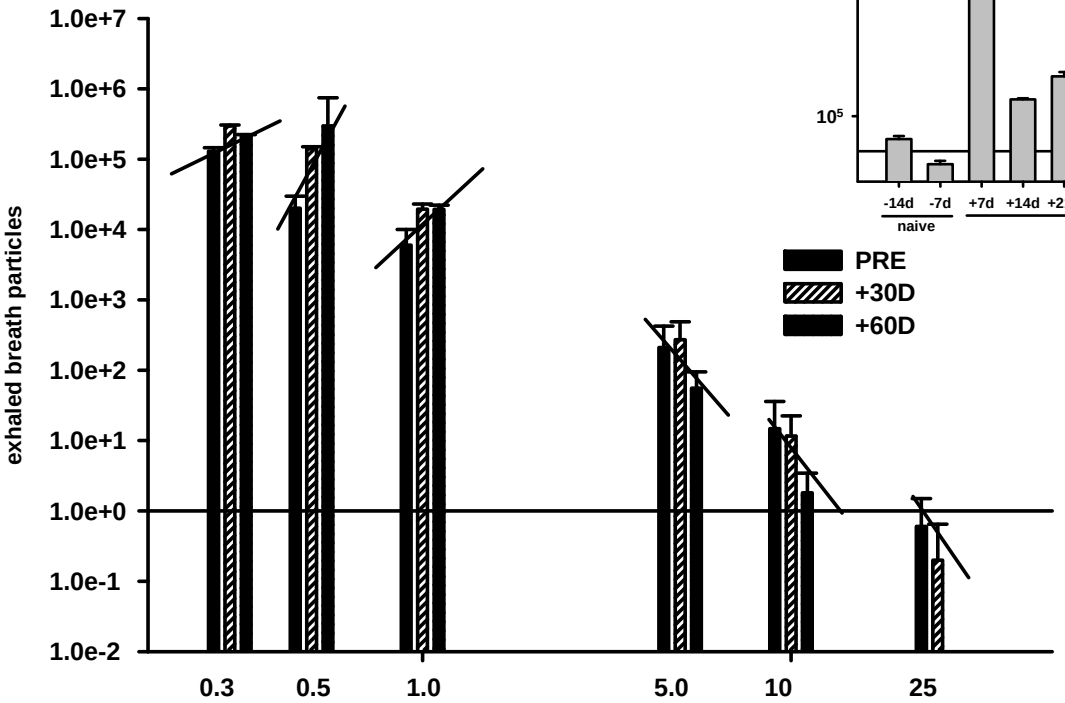
$$P = 1 - \exp\left[- \frac{(C - C_o) Iqt}{nC_a} \right]$$

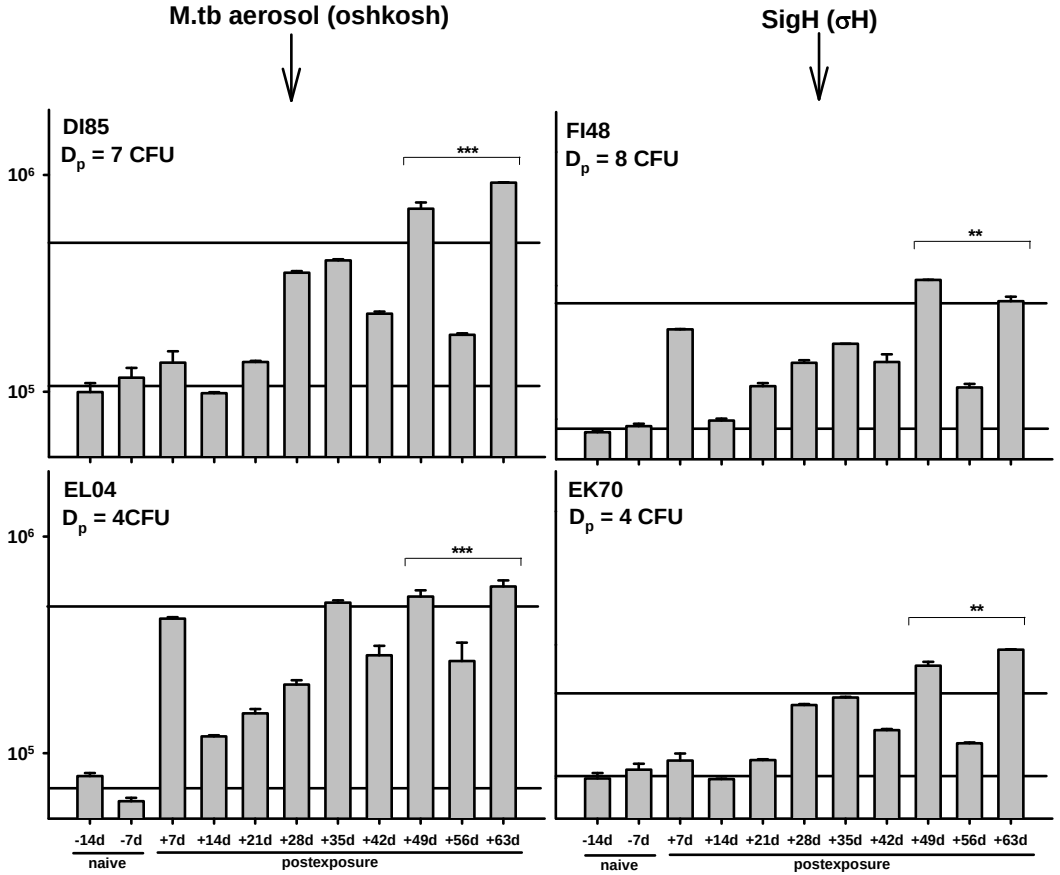
where P is the probability of infection for susceptible individual, I is the number of infectors, q is the quantum generation rate by an infected person, t is the total exposure time, n is the number of people in the ventilated space, C and Co are the average CO_2 concentration indoors and outdoors, respectively, and Ca is the CO_2 concentration added to exhaled breath during breathing.

- **host**
  - innate susceptibility
  - the **nature** and **number** of interactions with 'producers'
  - P is dynamic (too much so to model)
- **pathogen**
  - innate microbial fitness
  - source (from host)
  - particle aging/duration while in transit
  - dynamic size while in transit

temporal development of clinical tuberculosis

- the probability of exposure and 'infection' from in the context of naturally-generated aerosols
- dynamics of aerosol transmission





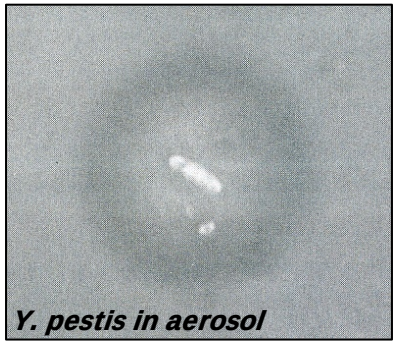
- significant parameters in temporal development of clinical disease
- physiological changes are induced in clinical *tb* (EBA production)

experimental aerobiological infection: noteworthy considerations

- **Microbial characterization**
  - microbial susceptibility in the environment
  - compensatory mechanisms of pathogens in stress environments
  - distribution from various generators
- **Physical characterization**
  - Particle size and heterodispersity
  - Multimodal distributions (environment and sythentic)
- **Initial deposition/interaction in the respiratory system**
  - Host-pathogen interaction in the respiratory system
  - innate response v. immune evasion mechanisms employed by some pathogens
  - Modeling aerosol-acquired disease in appropriate animal species
  - differential pathogenesis from exposure to distinct particle distributions

modeling airborne-acquired infection
source generation

airborne → droplet nuclei


Y. pestis in aerosol

droplet nuclei → viability

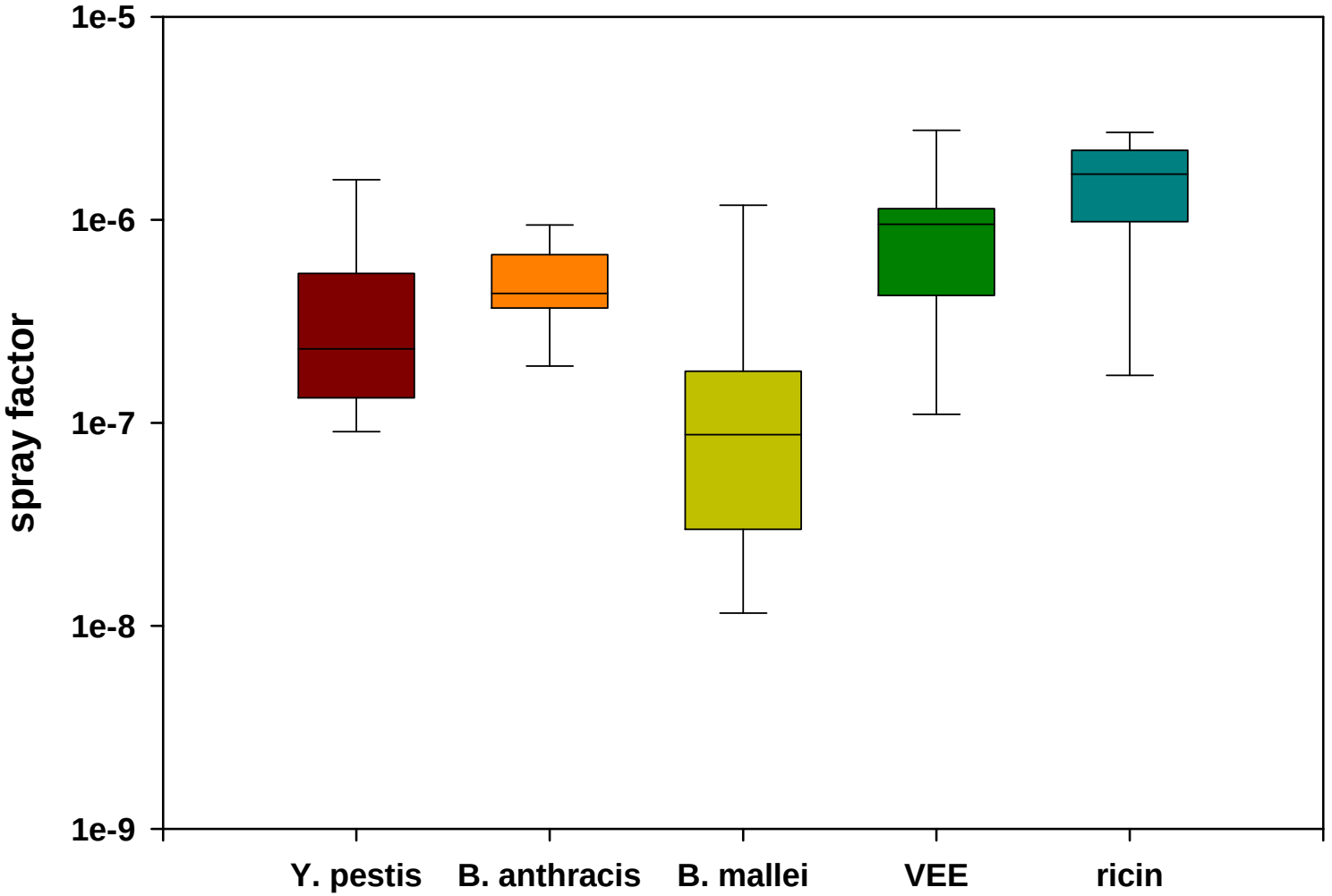
viability → pretreatment
viability → generation
viability → delivery

viability → infectivity

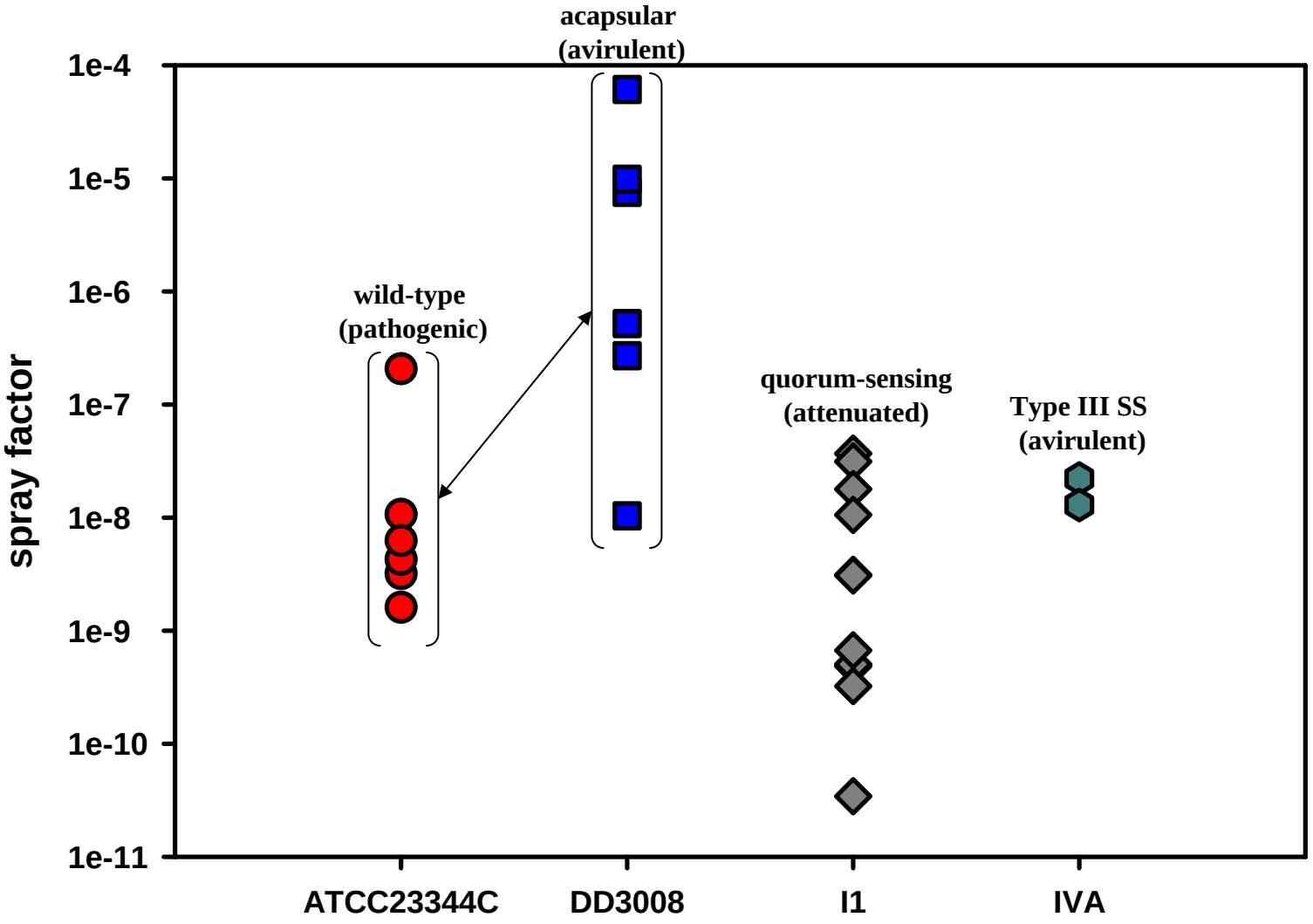
Organism	runs	relative vunerability*
E. coli	-	1.00
Bacillus globigii, vegetative	6	1.68
Bacilus globigii, spore	5	0.22
Bacillus smegatis	9	0.52
Strepococcus hemolyticus	4	0.97
Strepococcus viridans	13	0.93
Staphylococcus aureus	13	1.35
Bacteriophage	5	2.14
Influenza virus	-	1.36
Mycobacterium tuberculosis	-	0.84

Sample Efficiencies of Biological Threat Agents in Aerosol

Impact of Viability upon Estimated Aerosol Concentration

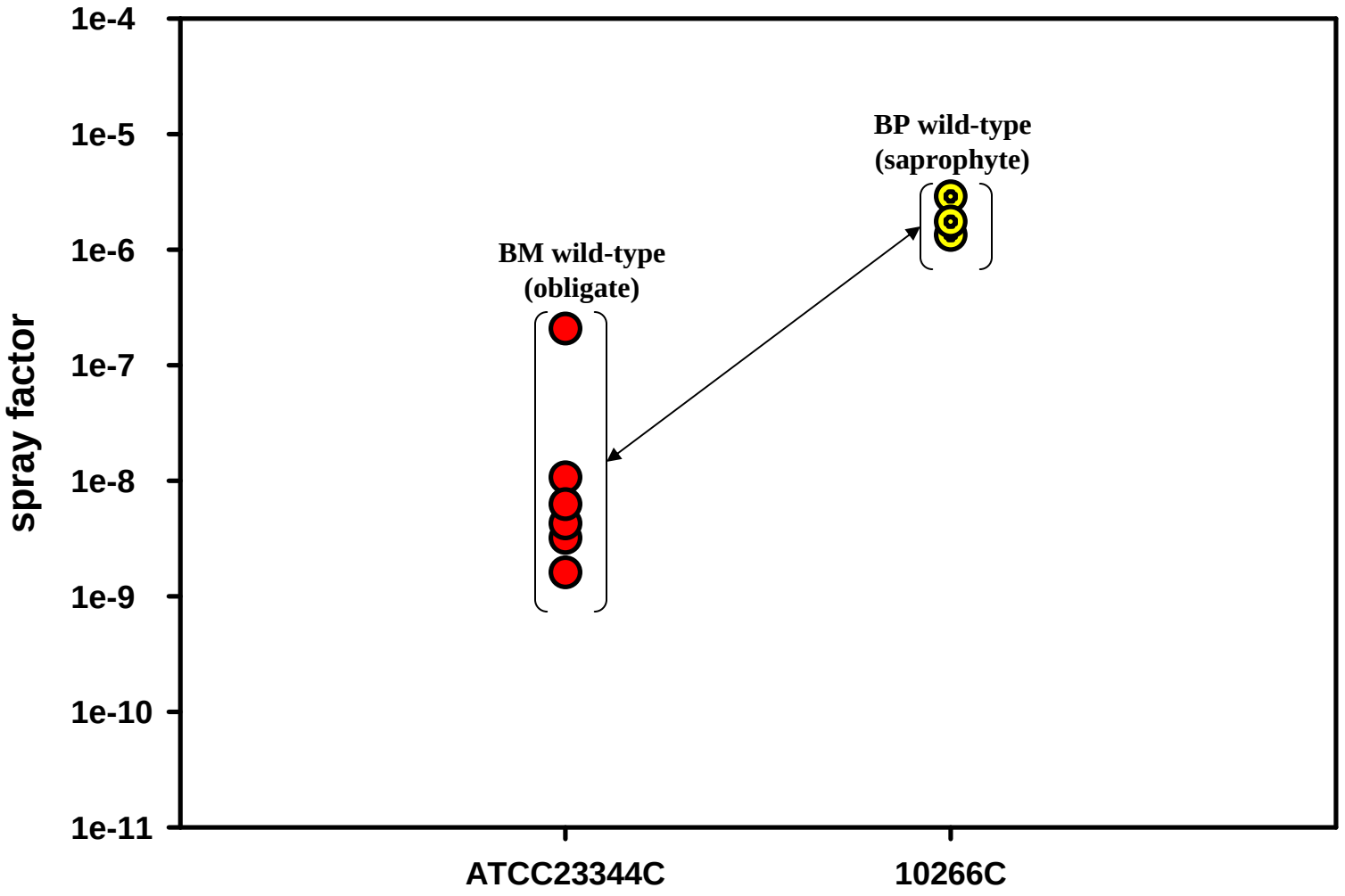


Viability Differences with a bacterial species *Burkholderia mallei*



Differences between genomically similar bacterial species

B. mallei v. B. pseudomallei

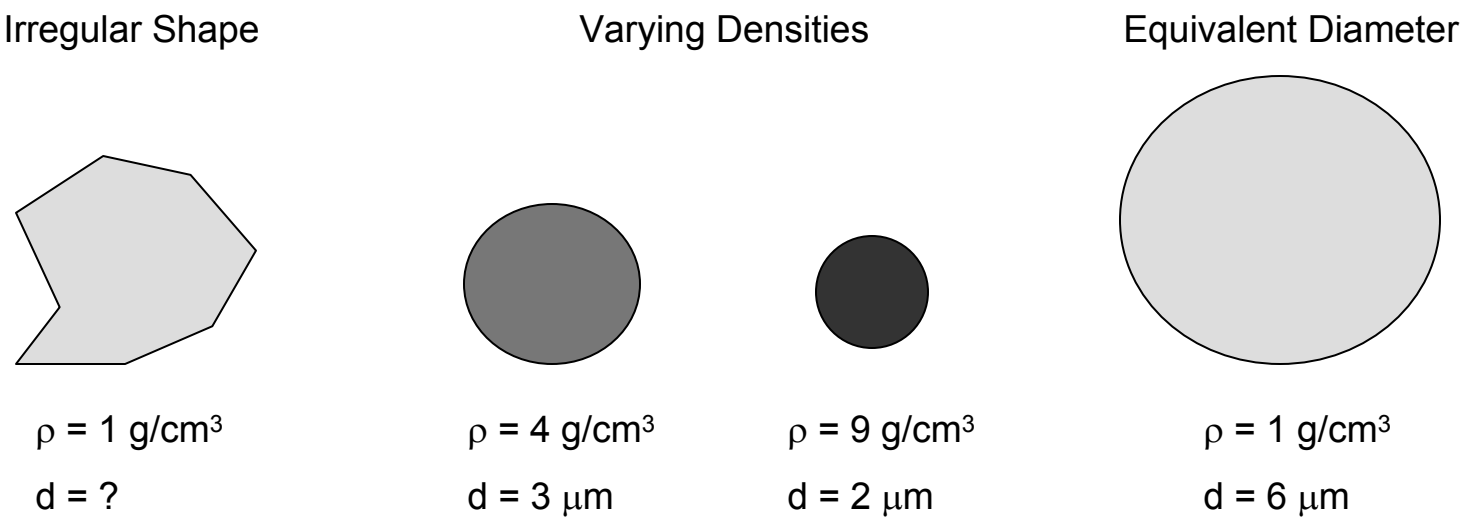


Aerosol biophysical characteristics

- Concentration
  - a function of the number and size of particles generated
- Particles characterized by:
  - geometric and aerodynamic size
  - shape, density and surface area
  - electrical charge / conductance
  - number and strength of interactions
  - between other particles or cloud components

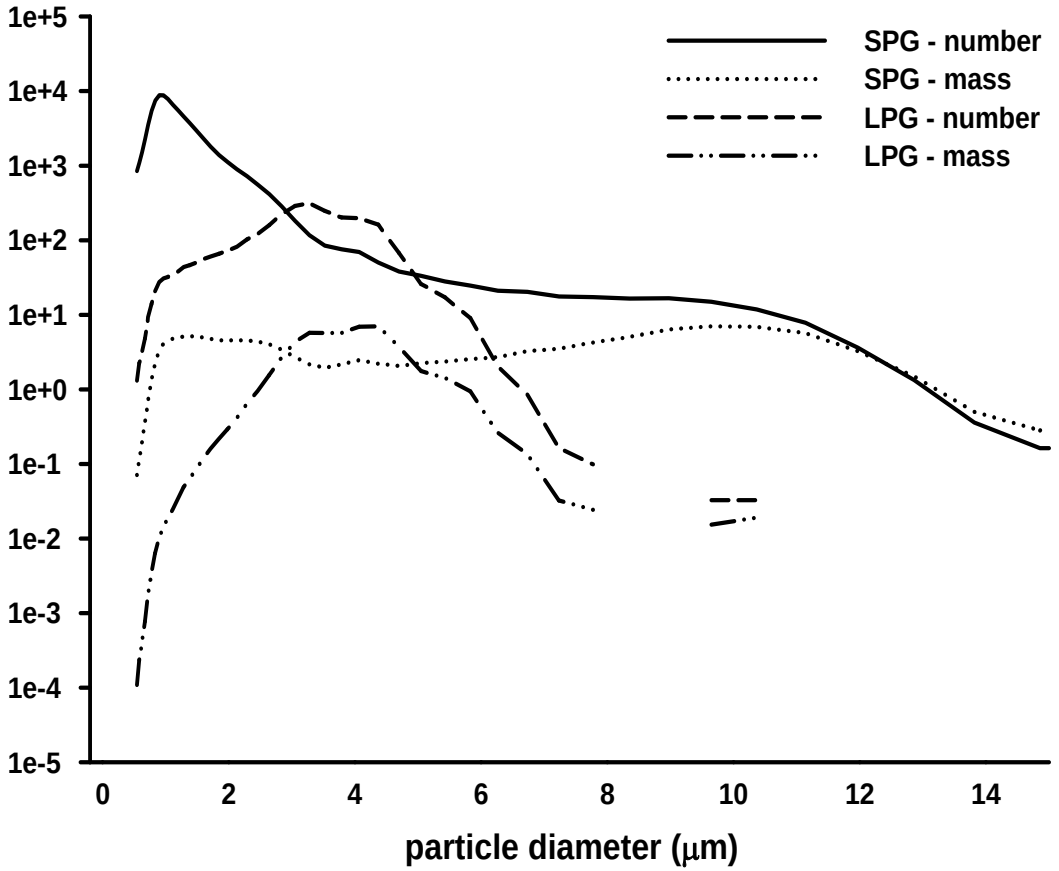
Biological Aerosol Size

- Use equivalent diameter that derives from particle property relevant to bioaerosol exposures
  - Mechanism of deposition
  - Particle size
- Aerodynamic diameter: diameter of a unit-density sphere having the same gravitational settling velocity as the particle being measured

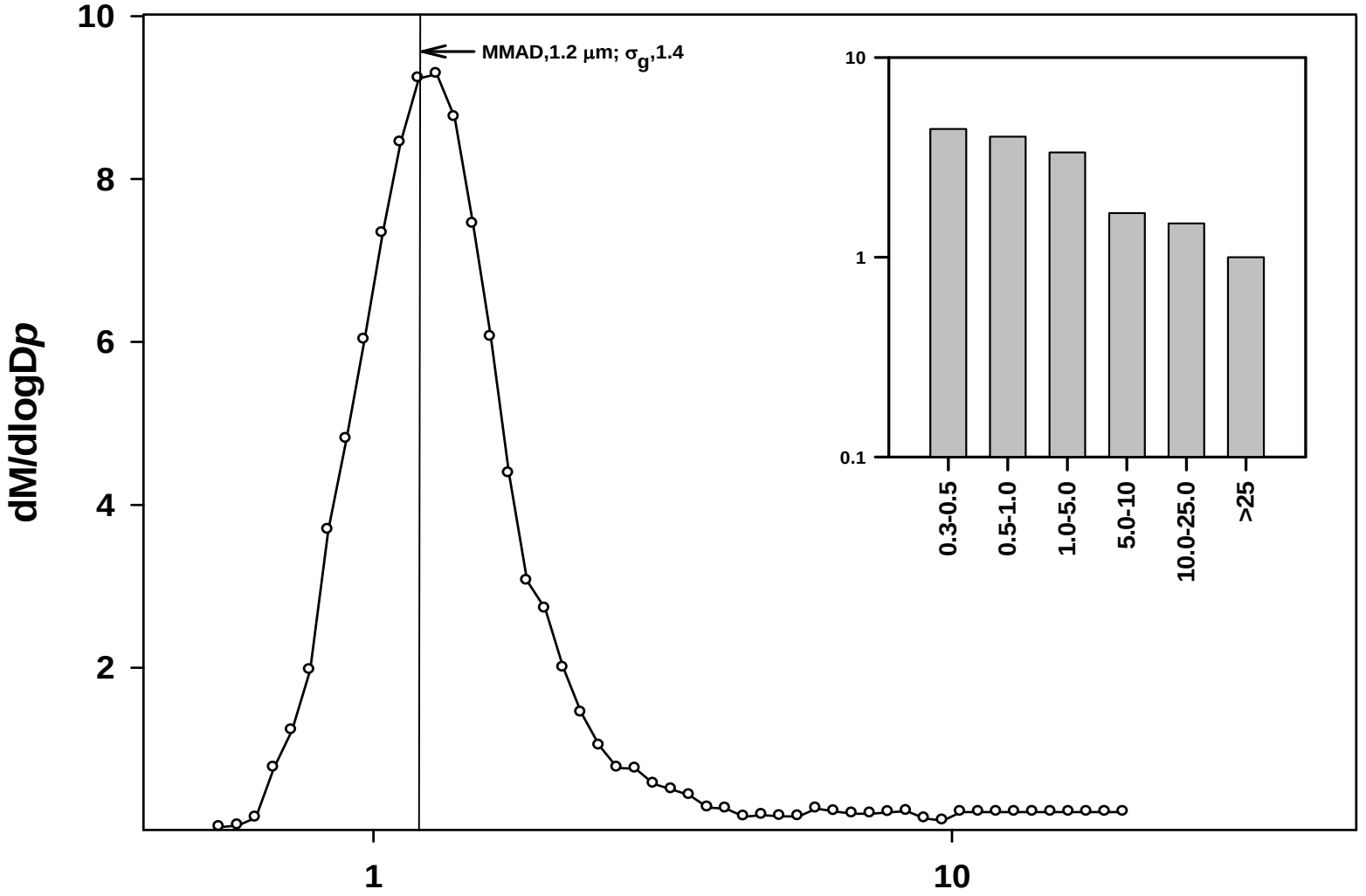


particle generation methods for infectious agents

- Standard generation methods employed for generating larger particle pathogen-containing aerosols **that retain viability**
  - spinning top aerosol generator
  - compared to standard industrial nebulizer and resulting distribution



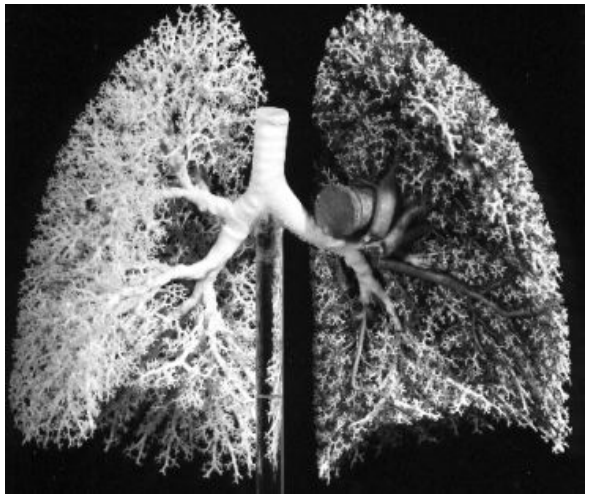
Source-Based Particle Distribution

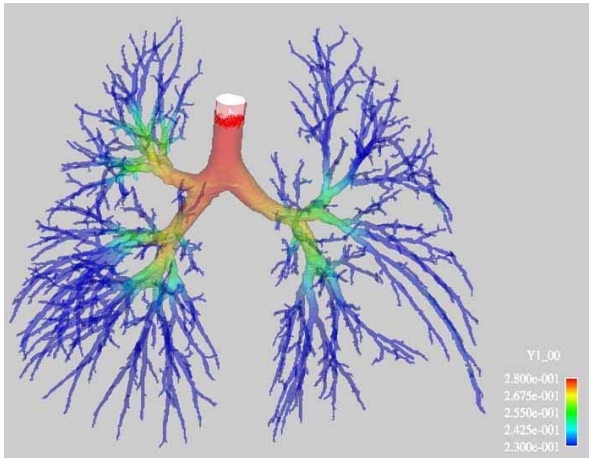


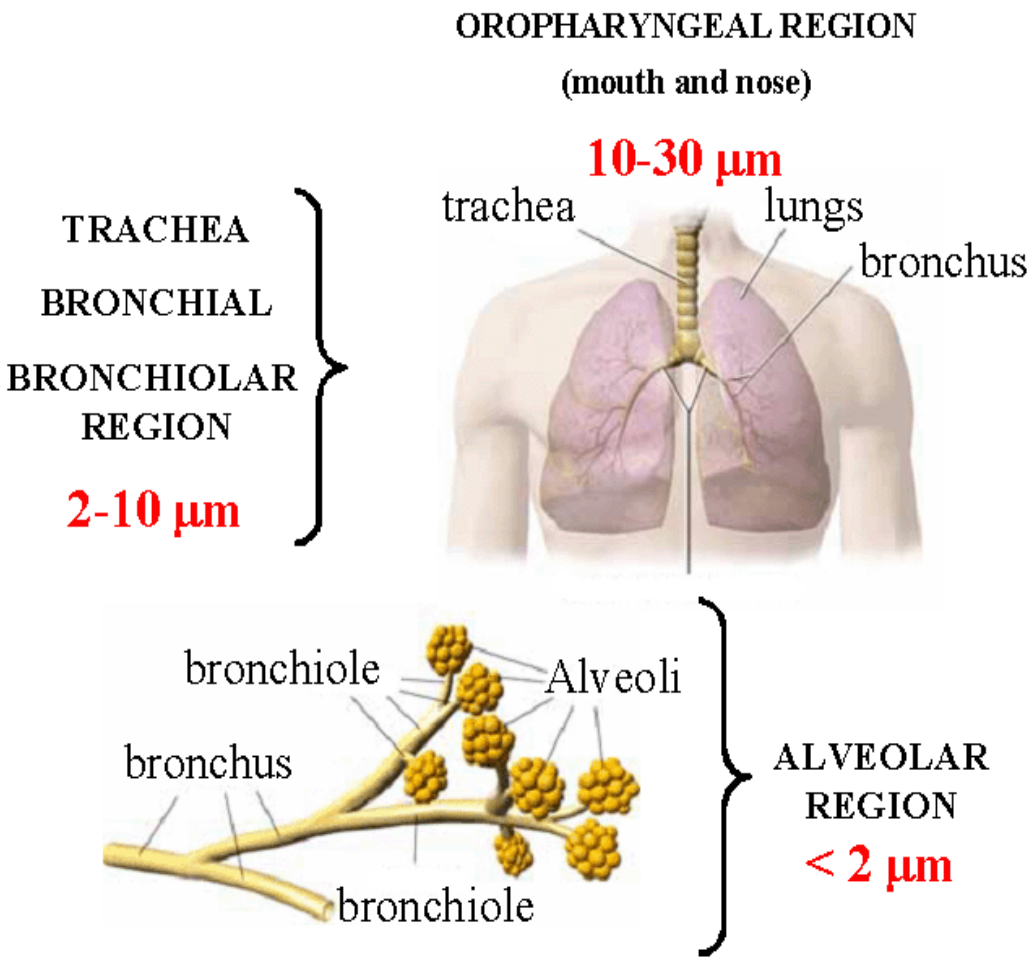
Initial Deposition and Clearance

- Particle deposition defines the organs/tissues with first contact
- Clearance defines the duration the body is in contact with the agent
  - bulk clearance
  - mucociliary clearance
  - alveolar clearance
- Ultimately both play major roles in the agents pathology and pathogenesis

Human deposition patterns



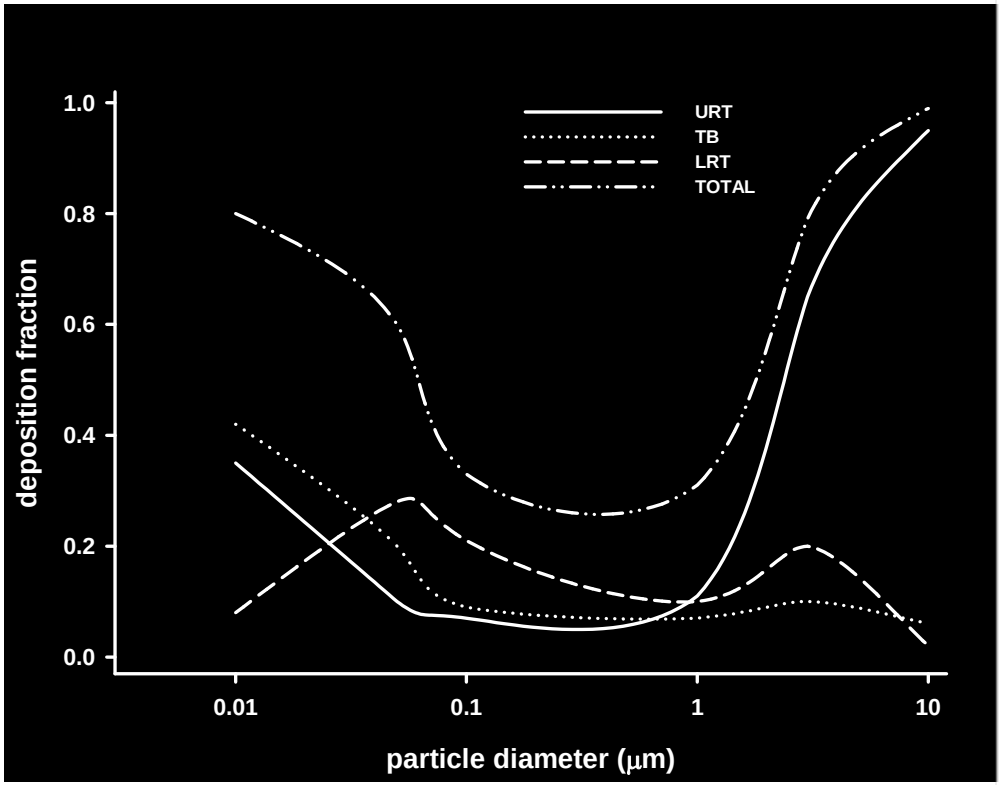


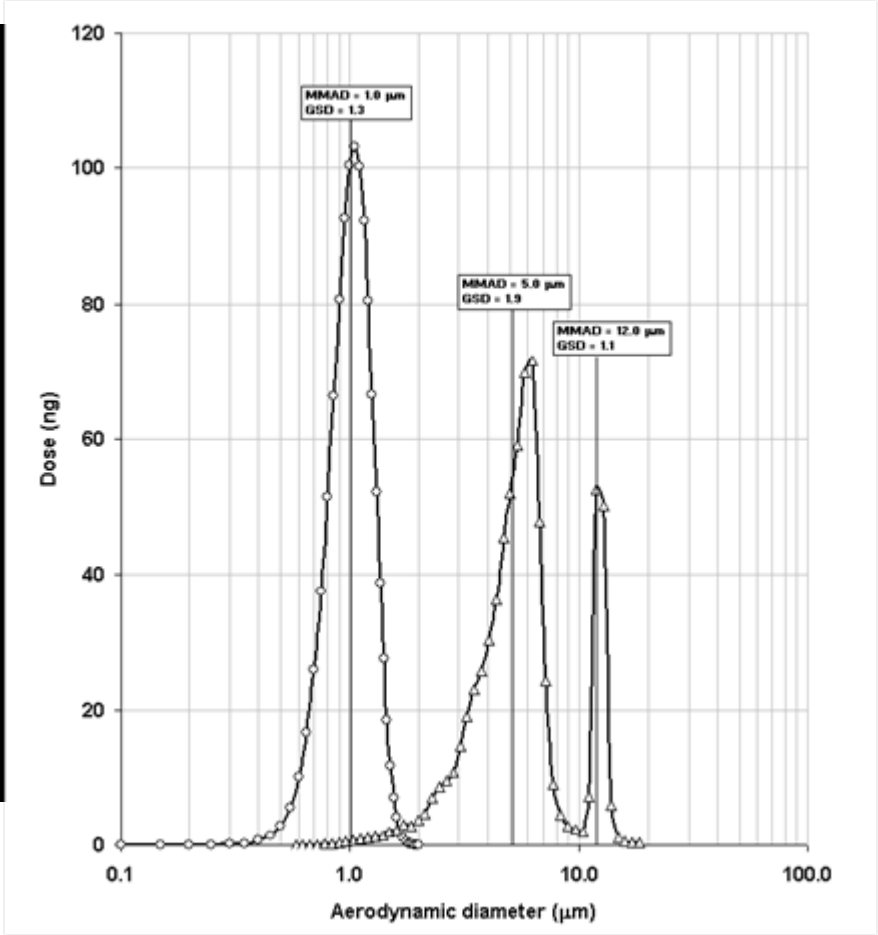


From ARL, PSU, 2007

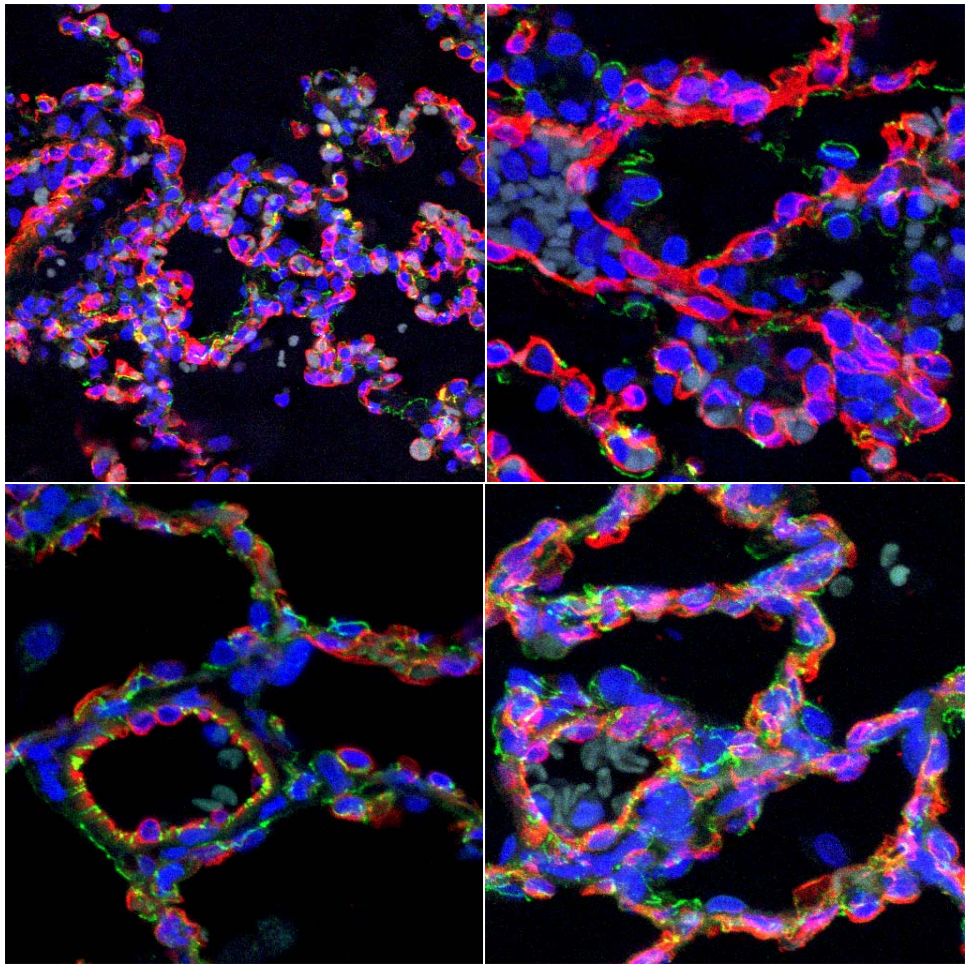
From Edwards *et al.*, 2009

Optimization of particle distributions





Initial host-pathogen interaction

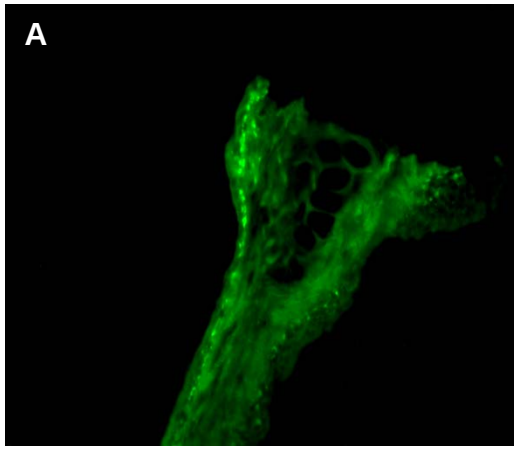


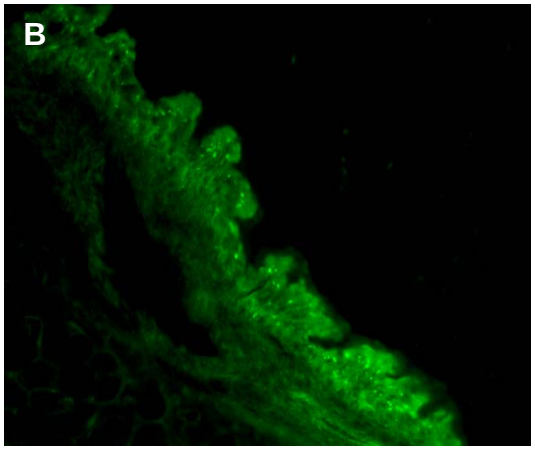
- Targeted tissues at the most susceptible portion of the respiratory tract
- Synthetically-prepared pathogen-containing aerosols take advantage of deposition into the LRT

agent/host response in mutimodal exposures

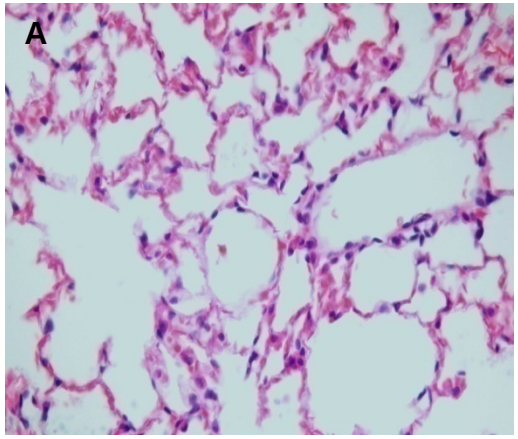
- Minimal database for understanding differences in host response from exposure to particle size
- regional differences in deposition
  - ↑ importance in locally-acting agents (e.g., ricin toxin)
    - primary endpoint → death
    - secondary endpoint → wt loss
  - ↑ importance in organ-targeting agents (e.g., alphaviral agents, EEE, VEE)
  - ↓ importance for agents that induce systemic, but not necessarily pneumonic disease state

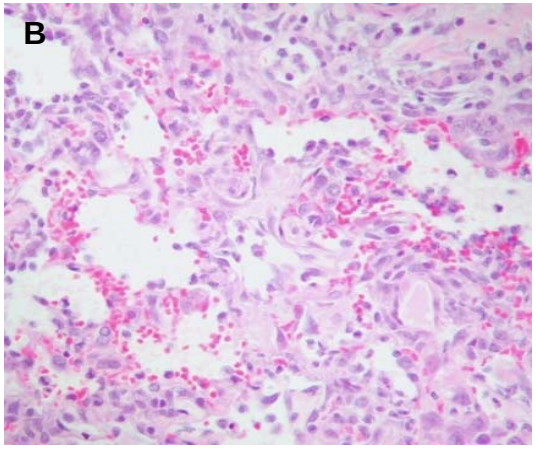
comparative pathogenesis: ricin toxin





Nasal turbinates (A) and olfactory epithelium (B) of a mouse exposed to 5 μm aerosols by whole-body chamber configuration. Epifluorescent ricin particles localized to the olfactory epithelium in the turbinates (A; 40X) whereas particles are localized to all levels of the olfactory epithelium (B; 100X).



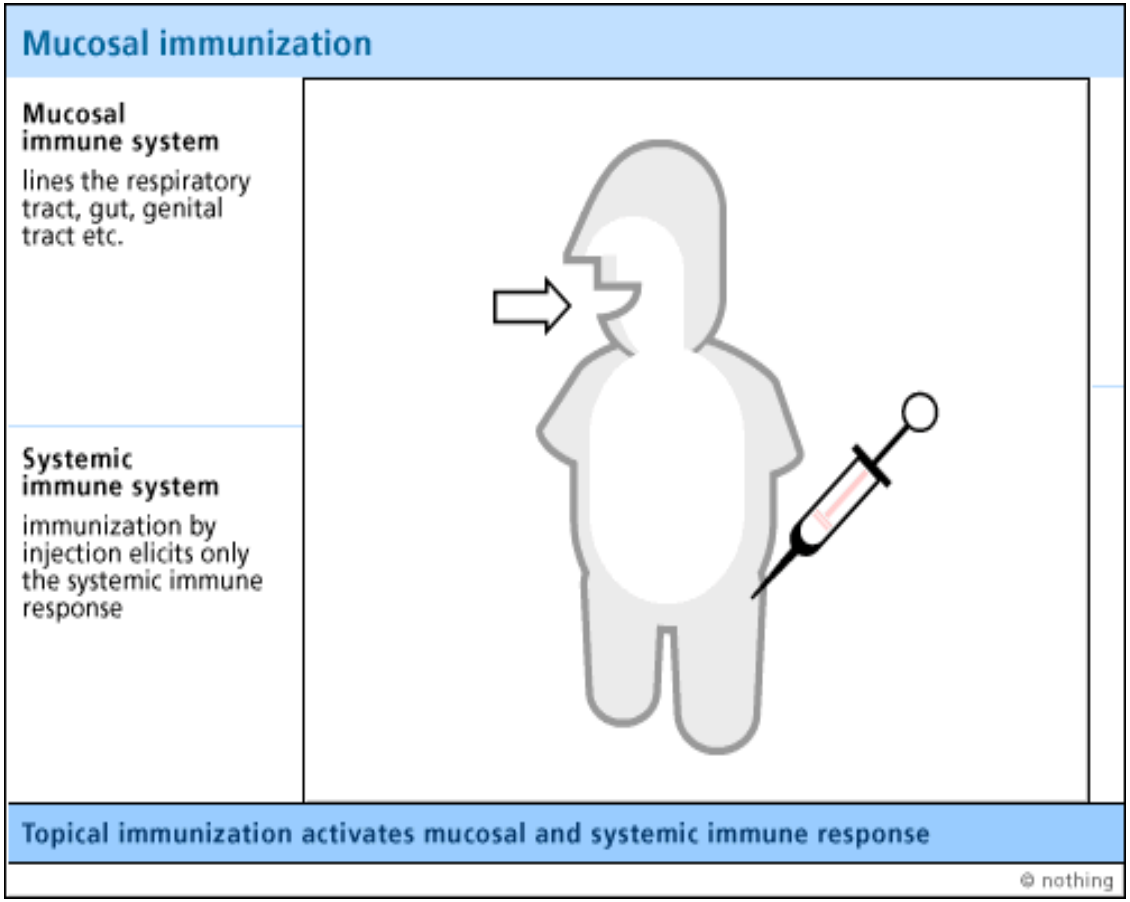


Lung section of mouse exposed to 5 μm ricin aerosols (A; 200X) or 1 μm particles (B; 400X). The lungs of the mouse exposed to the nonrespirable aerosol (A) shows no significant lesions. The lung of the mouse exposed to a respirable ricin aerosol (B) indicates marked interstitial pneumonia with alveolar edema, fibrin and hemorrhage.

from Roy et al., 2003

Advances is inhalation delivery (mucosal immunization)

- why?
  - scientific
    - concept of 'dual immunity'
      - elicits protective immunity
      - Needed for protection against enteric disease
      - Immunity at mucosal surfaces (route of entry)
      - Both serological IgG and IgA
    - equivalent seroconversion
    - Lower adverse advents
    - target-specific
    - potency
    - Rapidity of boost dosing
  - practical
    - self-administration
    - logistics
      - stockpile
      - holding temperature
    - reduction of healthcare personnel



Aerosol Vaccination Against Infectious/Toxic Agents
some recent (and not so recent) efforts

- 'biodefense' vaccines
  - anthrax[1], tularemia[2,3], VEE[3], SEB[7]
- other
  - Tuberculosis[6,7] diptheria[4], tetanus[5], measles[8,10], rubella[9,10]

[1] Aleksandrov et al., *Experiment of mass aerogenic vaccination against anthrax* (1959)
[2] Eigelsbach et al., *Aerogenic immunization of the monkey and guinea pig with live tularemia vaccine* (1961)
[3] Sawyer et al., *Simultaneous aerosol immunization of monkeys with live tularemia and live VEE vaccines* (1964)
[4] Muromstev et al., *Experimental reimmunization with diptheria toxoid by inhalation* (1960)
[5] Yamashiroya et al., *Aerosol vaccination with tetanus toxoid* (1966)
[6] Cohn et al., *Airborne immunization against tuberculosis* (1958)
[7] Tseng et al., *Humoral immunity to aerosolized SEB vaccinated with SEB toxoid-containing microspheres* (1995)
[8] Fernadez de Castro et al., *Measles vaccination by the aerosol method in Mexico* (1997)
[9] Ganguly et al., *Rubella virus immunization in pre-school children via the respiratory tract* (1974)
[10] Sepulveda-Amor, J. et al., *A randomized trial demonstrating successful boosting reaponses following simultaneous aerosols of measles and rubella (MR) vaccines in school age children* (2002)

Early Abandonment of the Effort

lack of advanced technology paired with suboptimal reagents

- early crude vaccines were reactogenic
- mainly live attenuated or toxoids used
  - adverse events ↑ over injection
  - no identified mucosal adjuvants
- Individual inhalation devices largely unavailable
- failure to identify ‘dual immunity’ concept
- troop compliance
  - was ‘cold chain’ logistical support up to the task?

Alternative Delivery: Inhalation
recent trends in biopharmaceuticals

- Therapeutics[1]
  - calcitonin (osteoporosis)
  - teriparatide (osteoporosis)
  - rGH (GH disorder)
  - interferon α (hepatitis C)
  - heparin (deep-vein thrombosis)
  - insulin (diabetes)
  - extendin-4 (diabetes)
  - α_1-antitrypsin (congenital emphysema)
- Vaccines
  - (EZ) measles
  - influenza

[1]Minter, B.A., Emerging Delivery Systems for Biopharmaceuticals, Decision Resources, 2001

Aerosol Vaccination for Measles and Rubella [1]

Acute Adverse Events (% incidence)

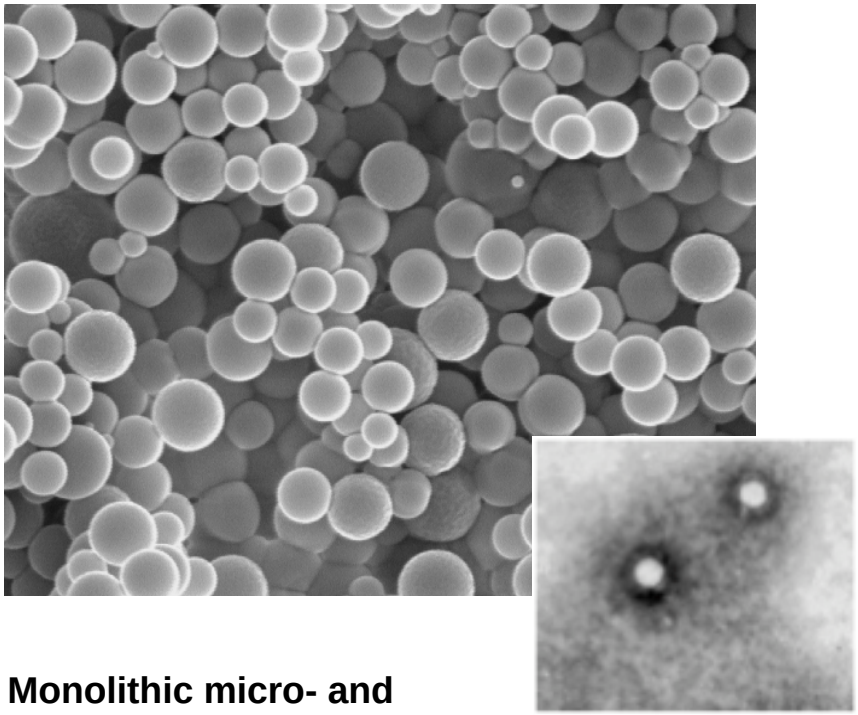
N	(307)	(225)	
Reactions	SC	AEROSOL	*P*
Fever	6.5	1.6	0.004
Rhinitis	3.3	0.4	0.02
Cough	17.2	0.4	0.0001
joint pain	4.9	0	0.0001
Diarrhea	1.3	0	0.4

Seropositivity/Seroconversion Rates (geometric mean)

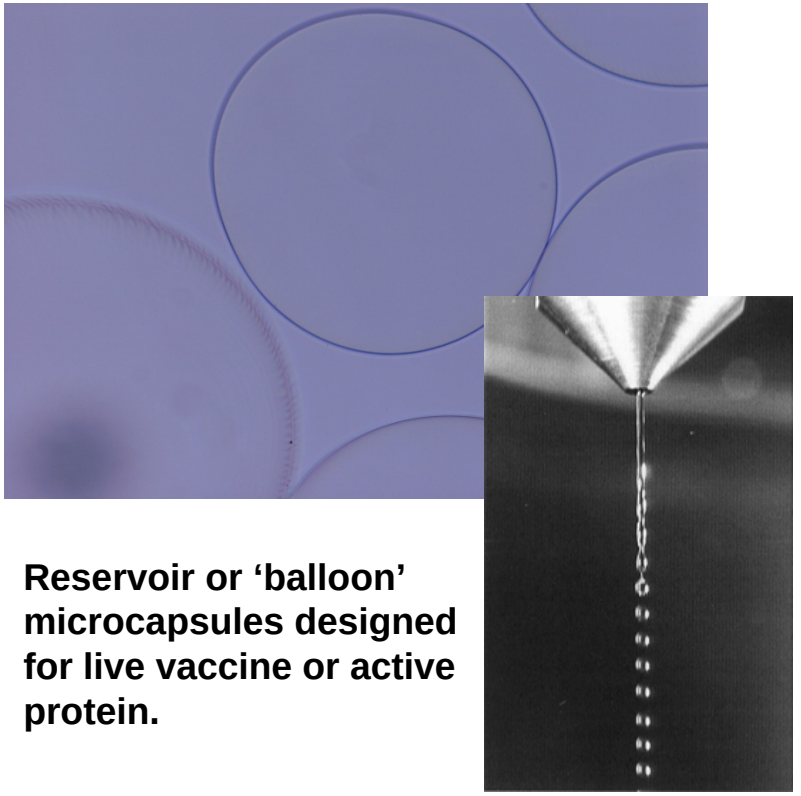
		SC	AEROSOL	*P*
Measles				
	PV seropositivity	99.7	98.8	0.04
	Seroconversion	55.1	52.9	0.6
	Ab titers	153.5	159.0	0.4
Rubella				
	PV seropositivity	92.2	99.6	0.001
	Seroconversion	82.4	98.8	0.001

[1]Data from Sepulveda-Amor, J. *et al*., 2002

Micro- and Nano-particle Vaccine Delivery Systems



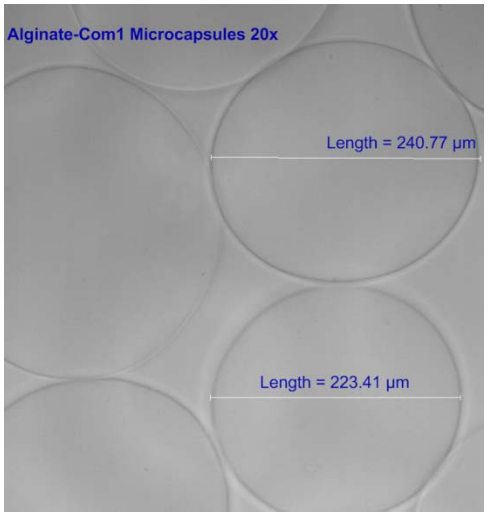
Monolithic micro- and nano-particles that are ideal for encapsulation of subunit or inactivated vaccine



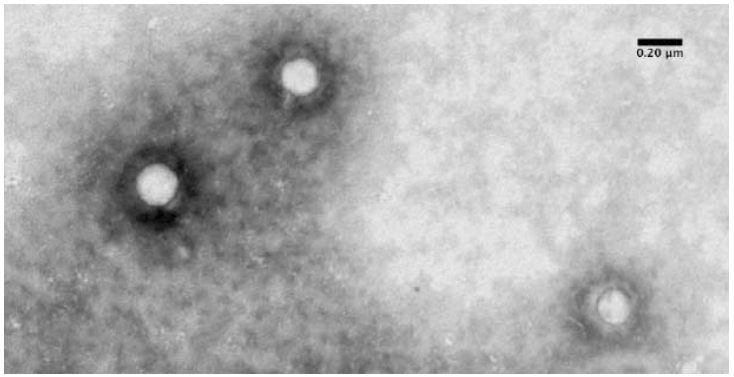
Reservoir or ‘balloon’ microcapsules designed for live vaccine or active protein.

Encapsulation Strategies: Oral and Intranasal Delivery

- Microcapsules: 200µm to 2000µm



- Nanoparticles: 50-300 nm



concluding remarks

- Aerosols and aerosol-acquired disease
  - Clear distinction between natural and experimental infection
  - Unique characterization of pathogen precedes optimized viability, size, and concentration
    - Demonstrative in focused animal studies
- Emerging technologies in biopharmaceutics that have facilitated the rapid development of specially-formulated inhalable biologics
- Recent proliferation in active development of inhalable biologicals continues to advance the science of microbially-active inhalable preparations