Aerosol delivery of MTB and Chemotherapeutics: Pitfalls and Plans

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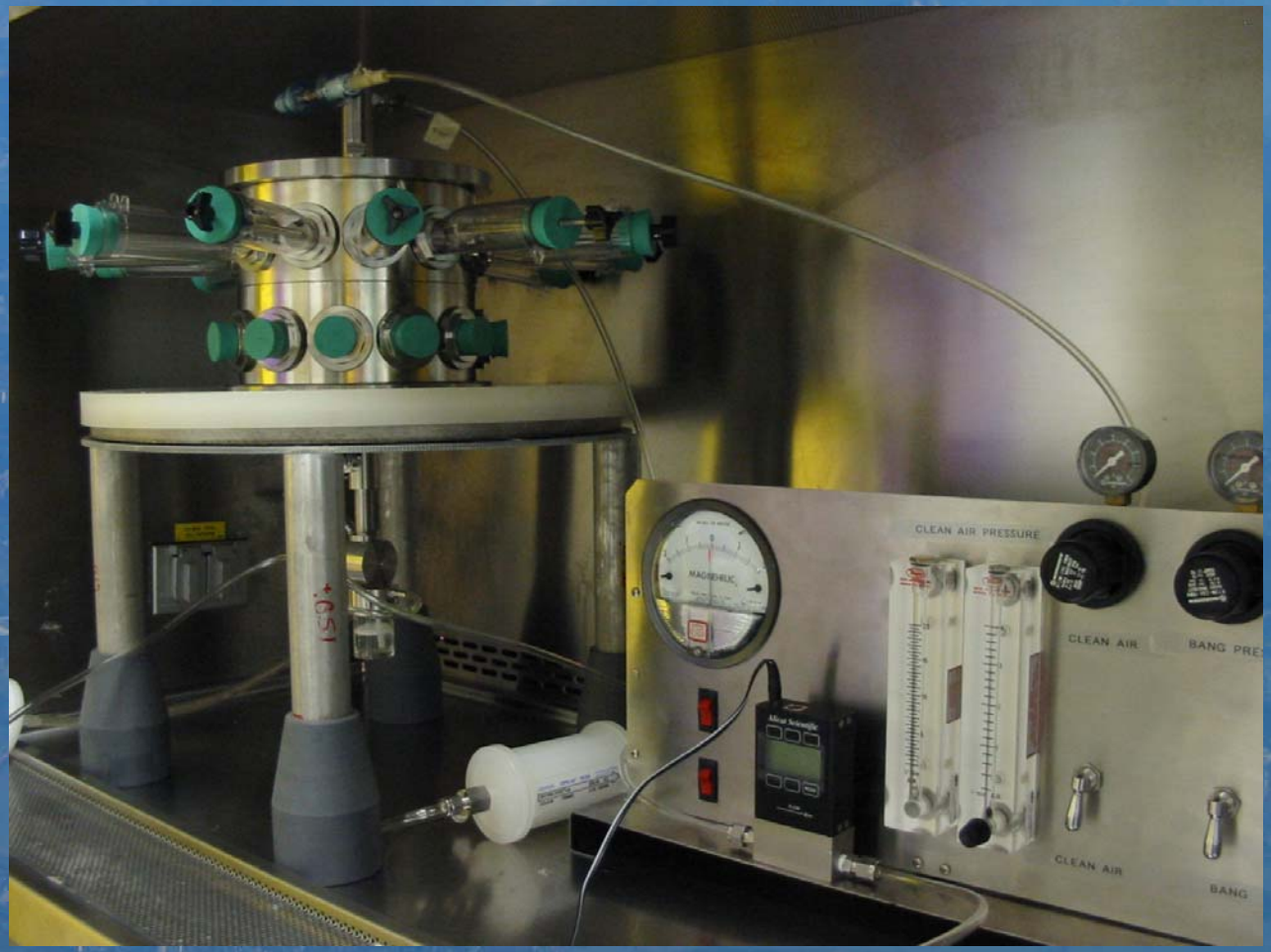
Nose only, directed-flow aerosol exposure unit with 24 mouse capacity per run

Our 2nd system in 4.5 years

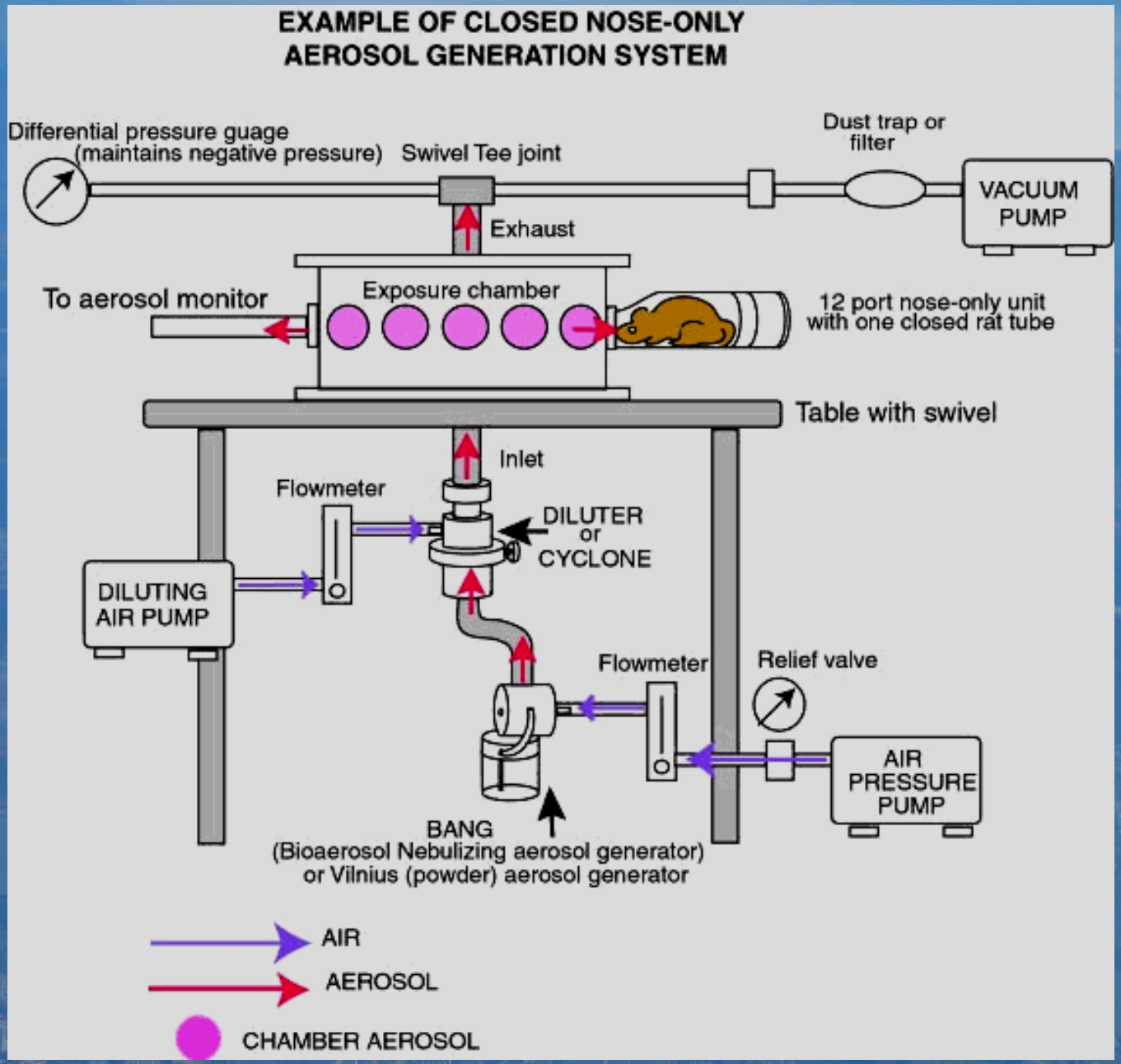
All stainless steel system with calibrated leak

Requires two pumps With positive air flow pump split between supply to BANG and dilution air

Had class III chamber, now running in Class 2B hood



CH Technologies, Inc., Westwood, NJ

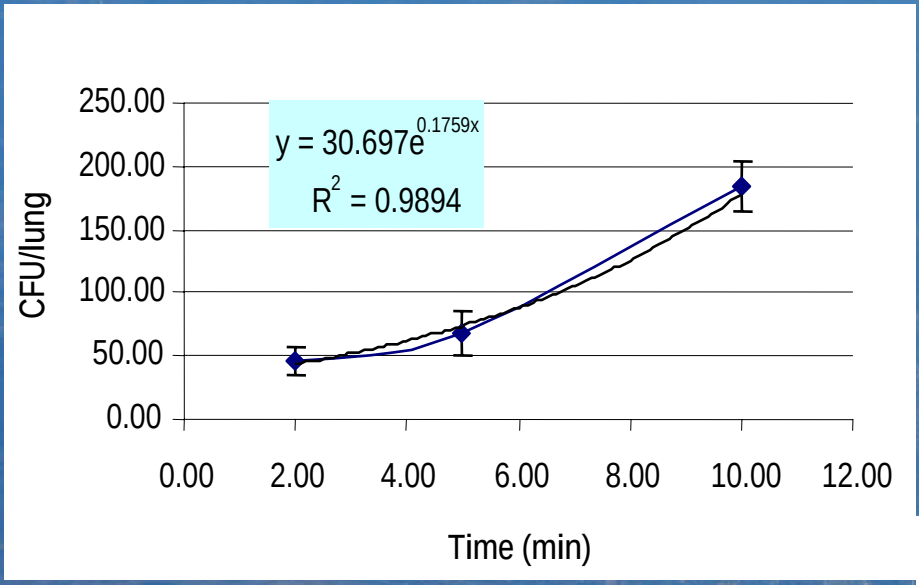


CH Technologies, Inc., Westwood, NJ

Equipment

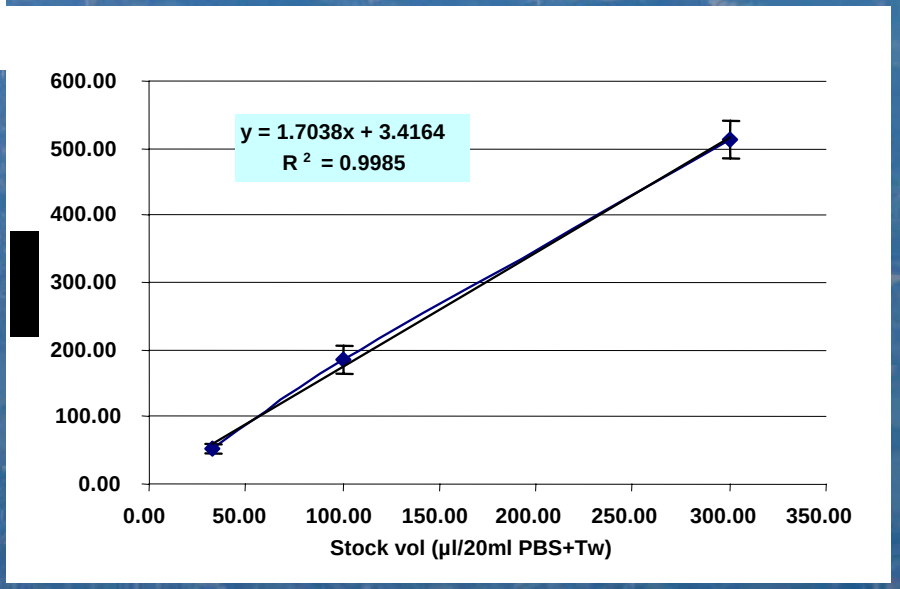
- BioAerosol Nebulizing Generator (BANG)
- Uses restricted positive air flow of 3 lpm when pushing clean air
- BANG flow 3 lpm during 10 minute exposure
- Vacuum maintained at 0.5 inch H_2O with calibrated leak

Calibration using *M. smegmatis* :

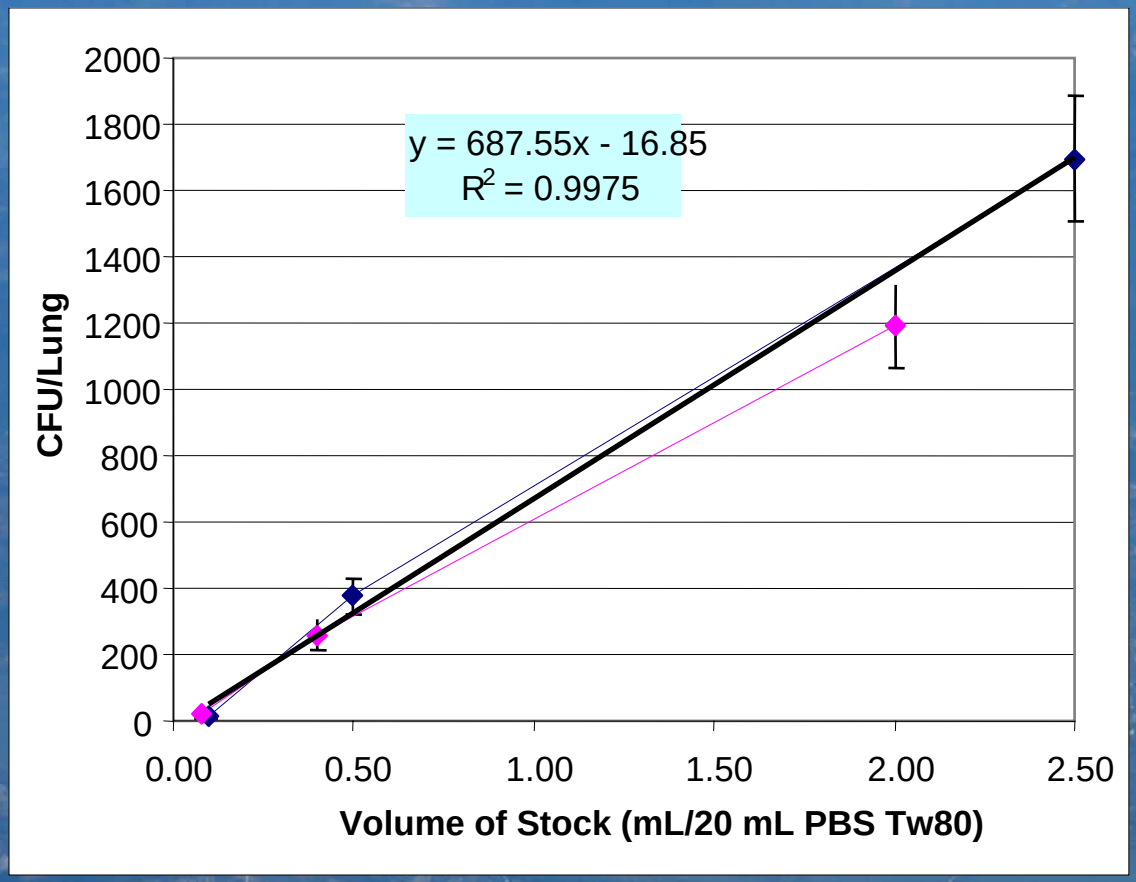


Culture A_{650} = 0.5
1/5 vol of 50% glycerol added and snap frozen
Stocks ~ 5 x 10^7 CFU after freezing

Stock diluted in PBS Tw80
Exposure time: 10 min
Stock volume : 65 ul in 20 mL

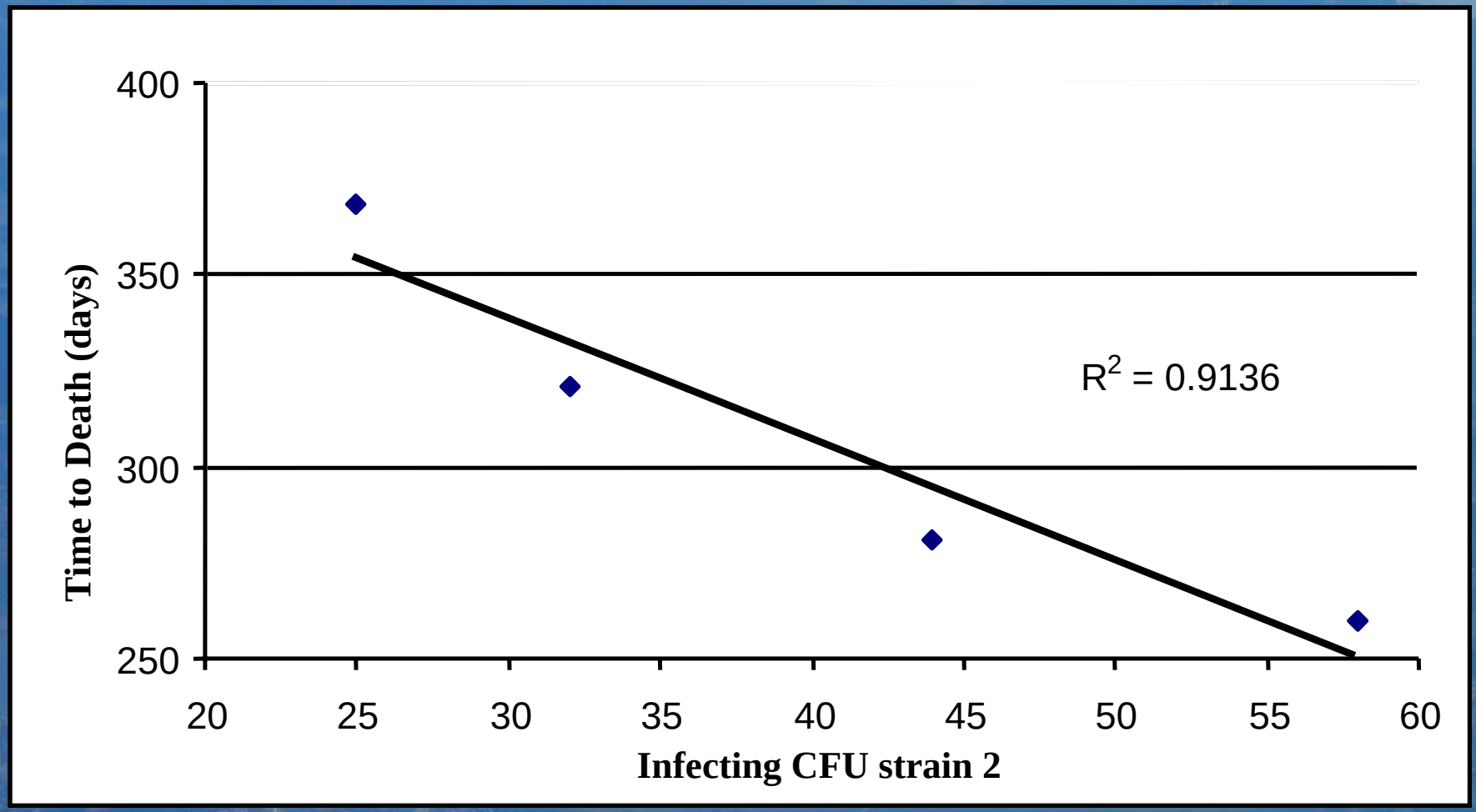


Calibration with *M. tuberculosis* H37Rv frozen stocks



Bacilli dilutions had equal CFU before and after 10 min BANG run

Infecting CFU of strain 2 vs median time to death in C57Bl/6 mice



Conclusion: time to death is proportional to number strain 2 bacteria present in lung early in infection

Practical Considerations

- Number of animals to be infected/treated
- Weight and size of system
- Design of cabinet
- Frequency and method of decontamination of system
- Method of maintaining system
- Sampling from system
- Calibration and standardization

What do we mean by latency/persistence in tuberculosis?

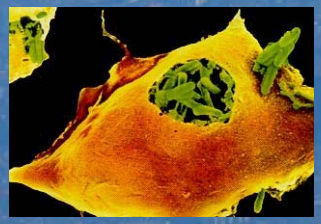
exposure

70%

30%

no infection

infection



60%

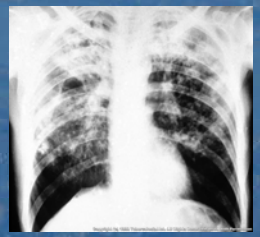
40%

latent TB

active TB

healthy
2-23%/lifetime

HIV
5-10%/year

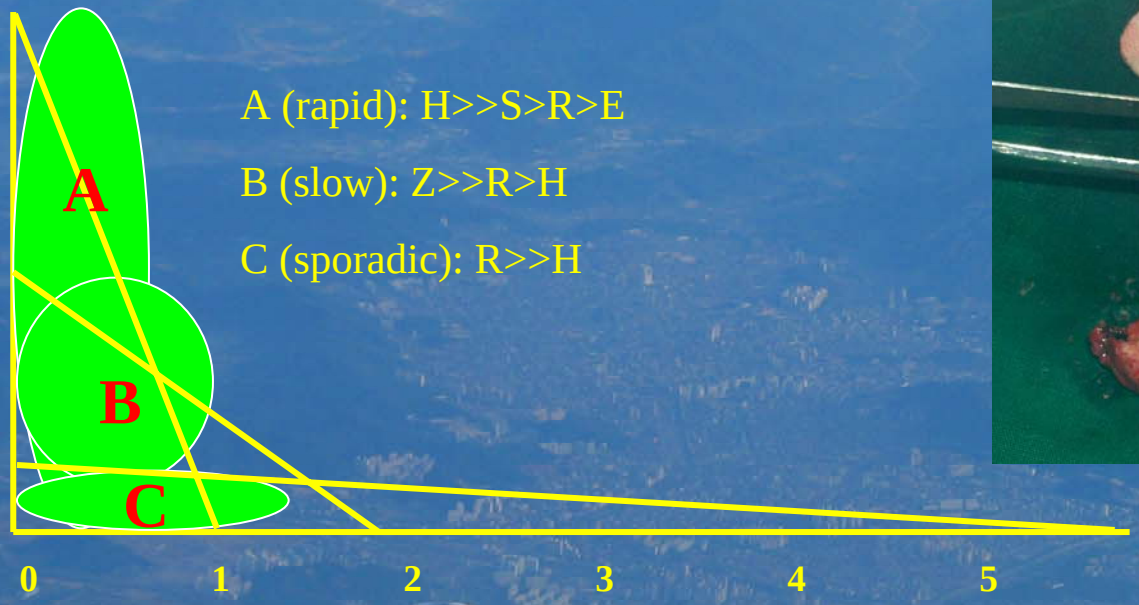


8 Million cases
Of Active TB

2-3 Billion
Cases of
“Latent” TB

Why does TB chemotherapy require 6 months?

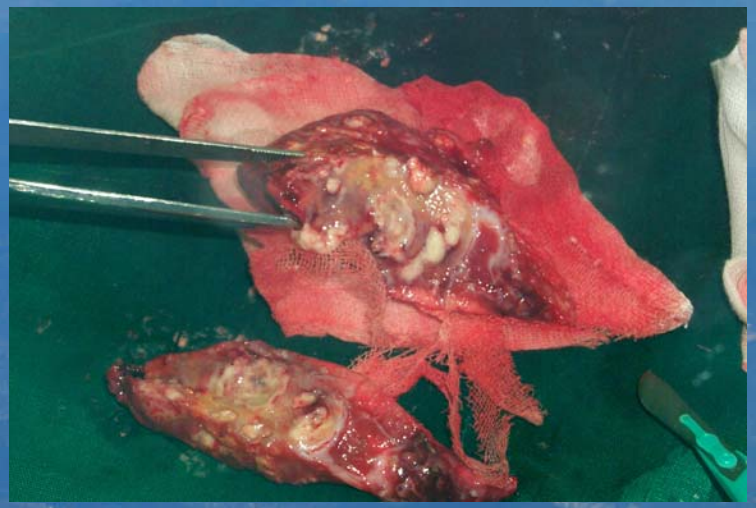
- Mitchison Model for TB Chemotherapy (Bactericidal (A) vs Sterilizing Effects(B,C))



A (rapid): H>>S>R>E

B (slow): Z>>R>H

C (sporadic): R>>H



Mitchison Model implications

1. Existence of metabolically distinct populations of TB in a host.
2. Potential variation in sensitivity of these populations to antimicrobial efficacy
3. Length of therapy is entirely dependent upon eradication of the “persistor” phenotype

Current Pharmacotherapy

- Based on BMRC trials
- Intensive phase: HRZ (+/-E or S)
- Continuation phase: HR x 4 mo (intermittent 2-3 x/wk)
- Current WHO Guidelines:

Patient	Intensive Phase	Continuation Phase
Smear + TB,	2 of EHRZ or 2 of SHRZ	4 of HR
Smear – Pulmonary TB,	2 of EHRZ or 2 of SHRZ	4 of H_3R_3
Extra pulmonary TB	2 of EHRZ or 2 of SHRZ	6 of HE

Time variations of treatment regimens

Hypothesis: In tuberculosis, mycobacteria with physiologic privilege as defined by temporally induced **drug tolerant** states exist

Experimental Design:

90 C57/Bl6 mice infected with approx. 100 bacilli

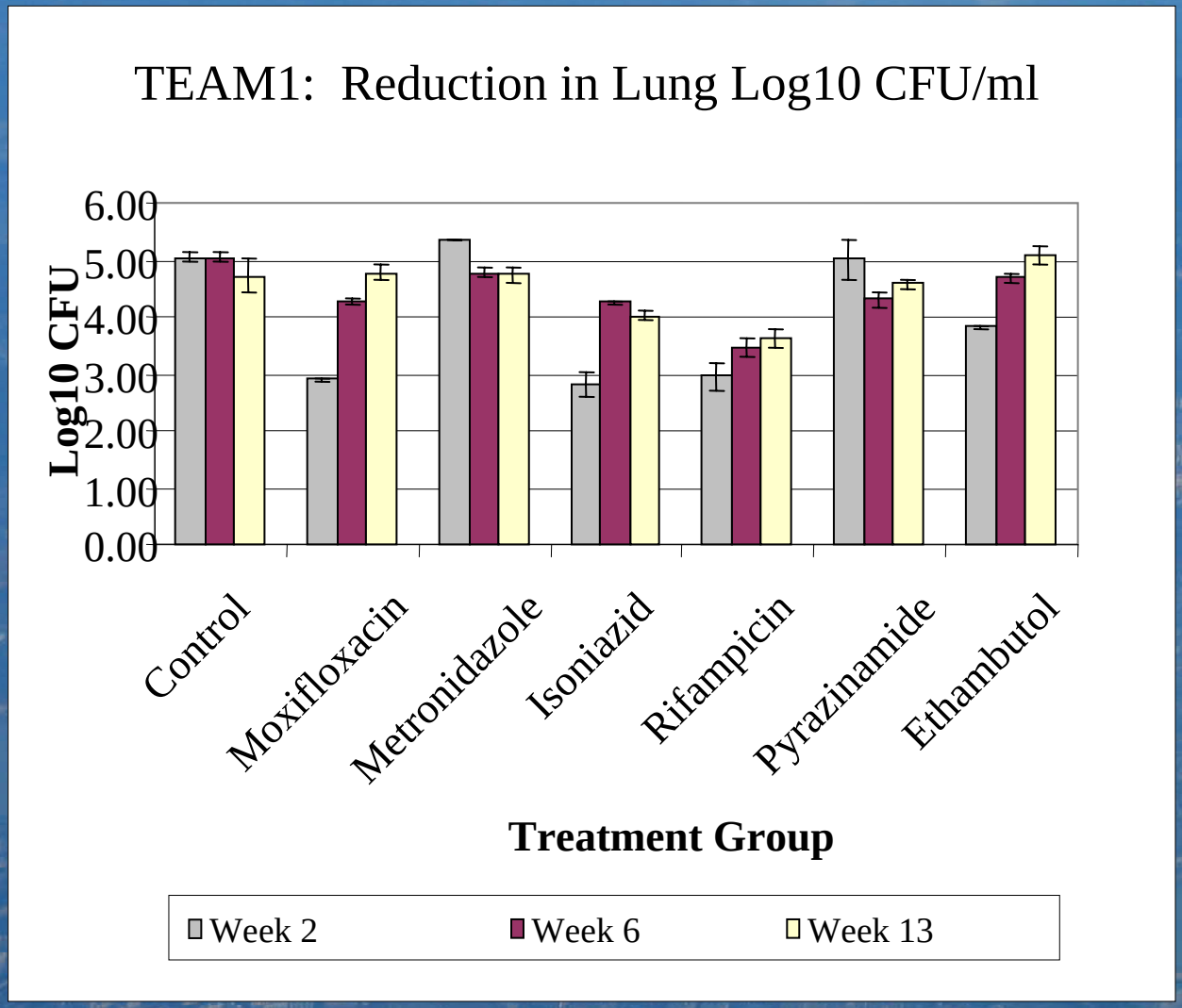
- Wk 2: therapy initiation
  Harvest after 2 wks of Rx
- Wk 6: therapy initiation
  Harvest after 2 wks of Rx
- Wk 13: therapy initiation
  Harvest after 2 wks of Rx

Treatment Groups (gavage):

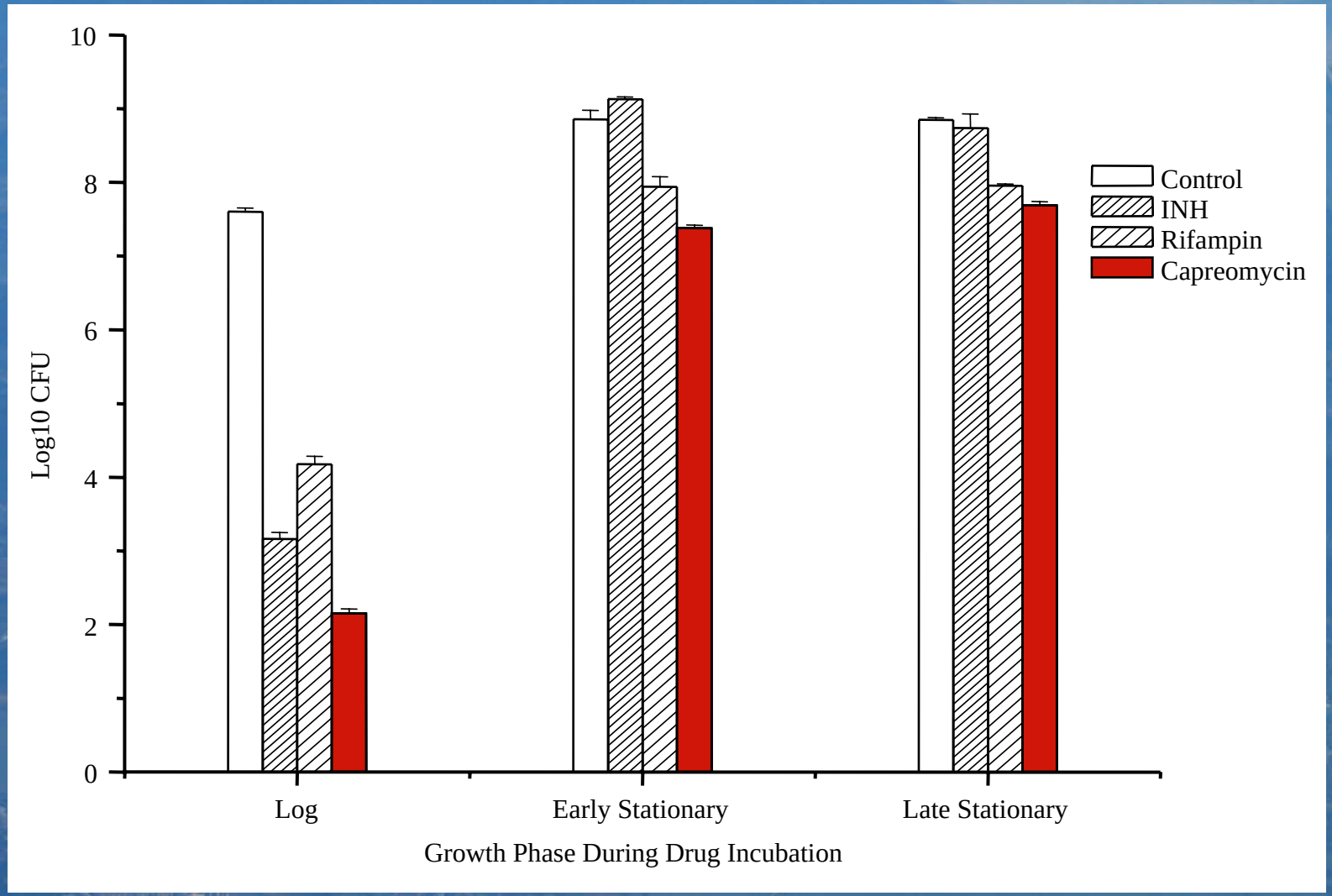
1. INH (25 mg/kg/d)
2. Ethambutol (50 mg/kg/d)
3. Pyrazinamide (150 mg/kg/d)
4. Rifampicin (25 mg/kg/d)
5. Moxifloxacin (100 mg/kg/d)
6. Control: no treatment

Endpoints: 1. Granuloma 2. CFU

Temporal Efficacy: Exp 1 Lung CFU



In Vitro Efficacy: Comparison of Growth Phase Tolerance



Can direct delivery of antituberculars to the lung reduce bioburden better than gavage or injection?

Hypothesis: There exists heterogeneity of mycobacteria with pharmacologic privilege as defined by access of Rx.

Experimental design:

C57/BL6 infected with 100 bacilli/mouse

↓

14 days

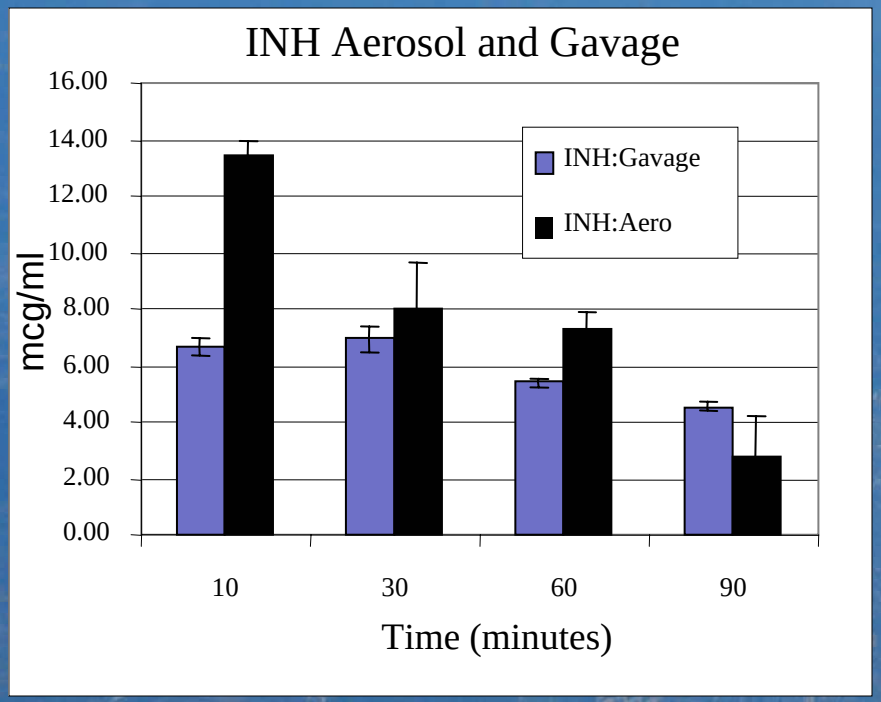
Drug Treatment (14 days) — Control: No Treatment

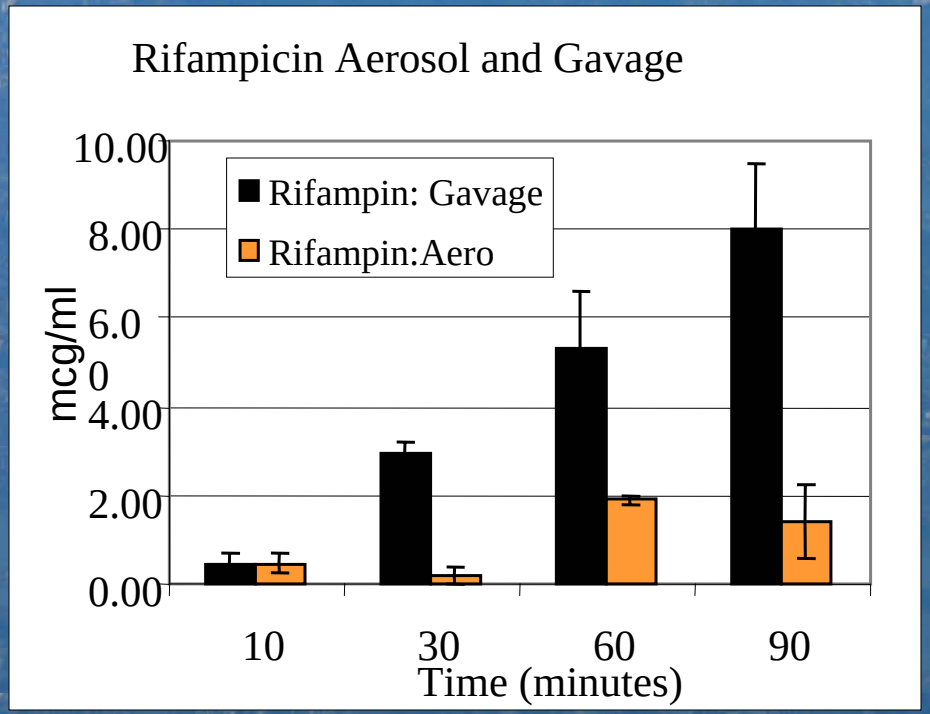
- Aerosol (conc x 30 min/d)
- Subq injection (200mg/kg/d)

Harvest and compare

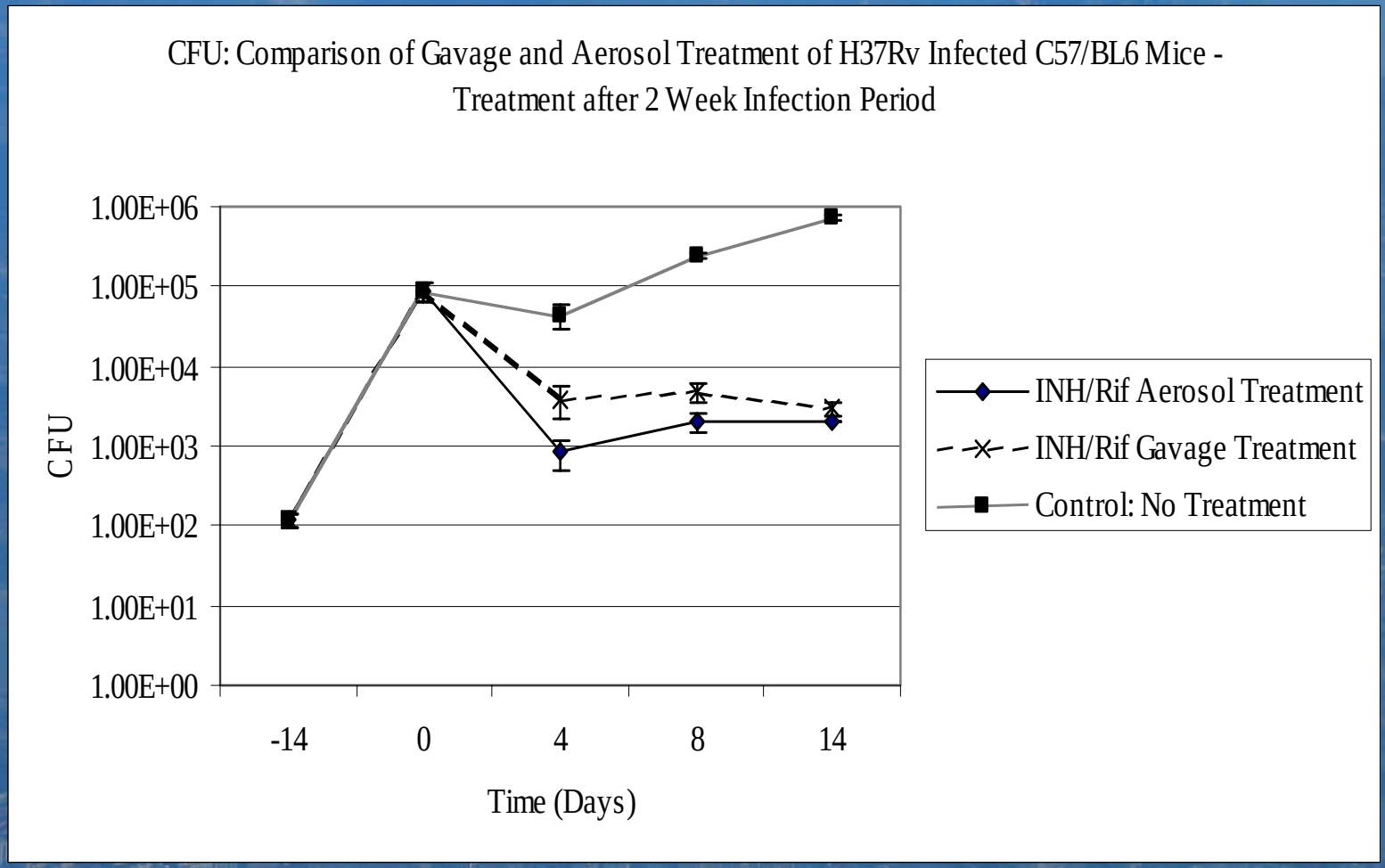
Endpoints: 1. Granuloma count, 2. CFU

Proof of Principle: PK Matching with Treatment Efficacy

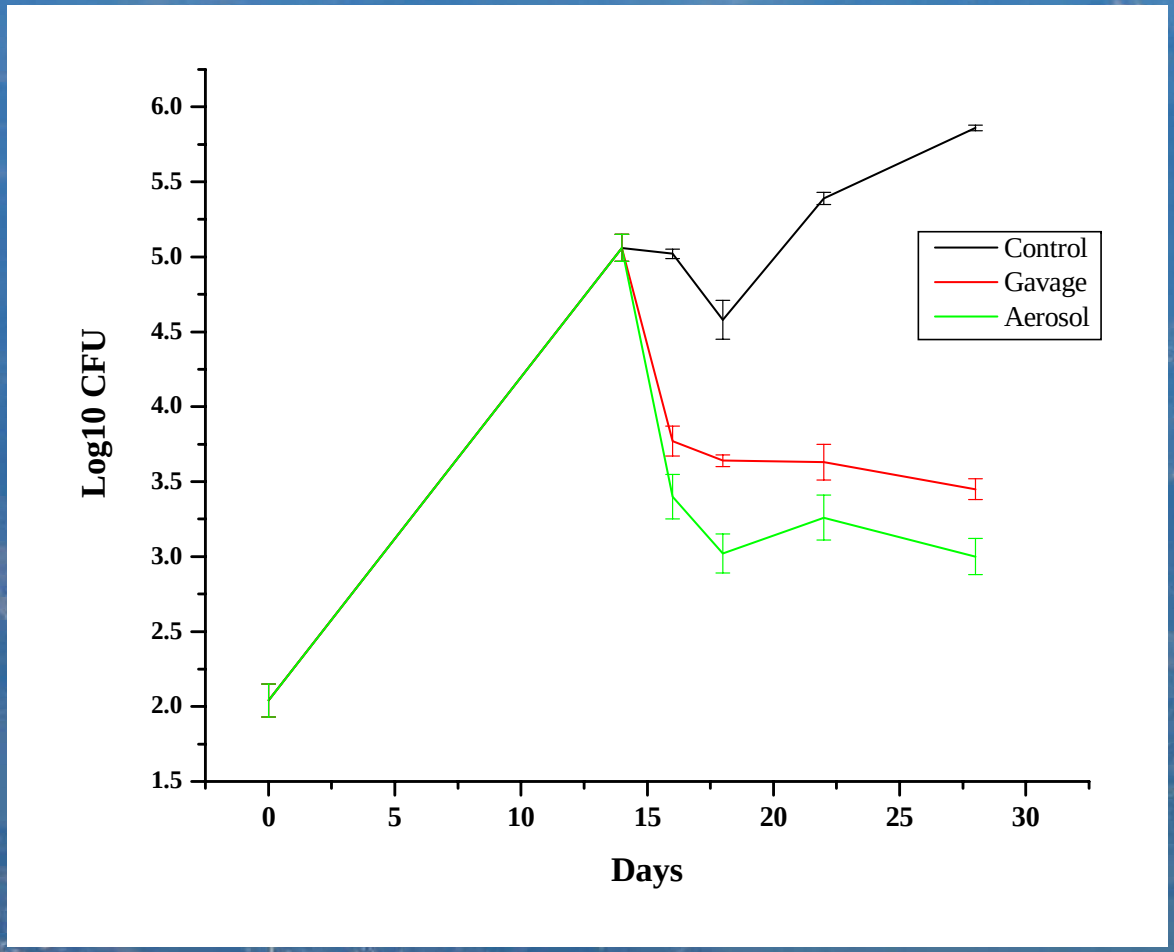




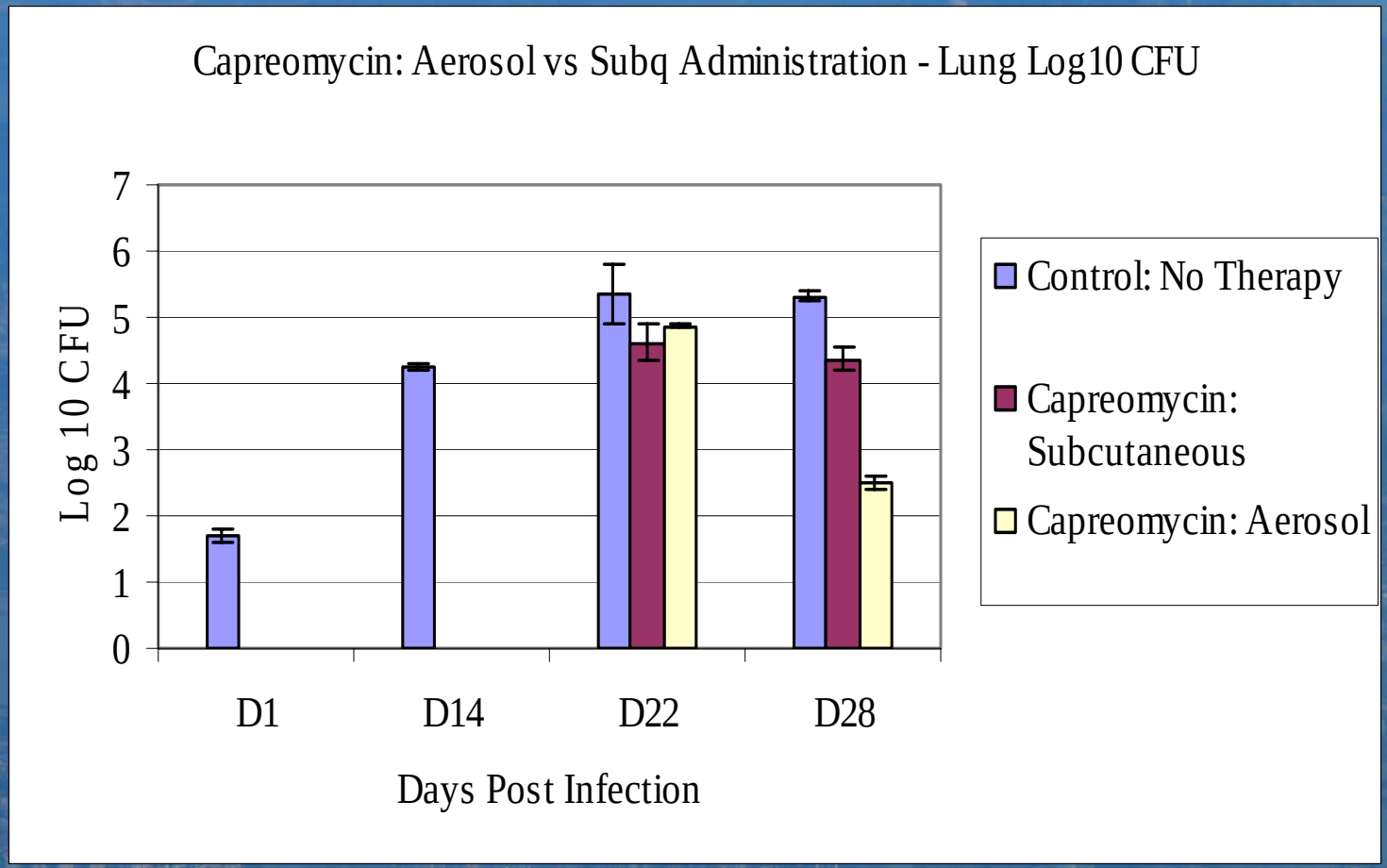
INH/Rif Aerosol



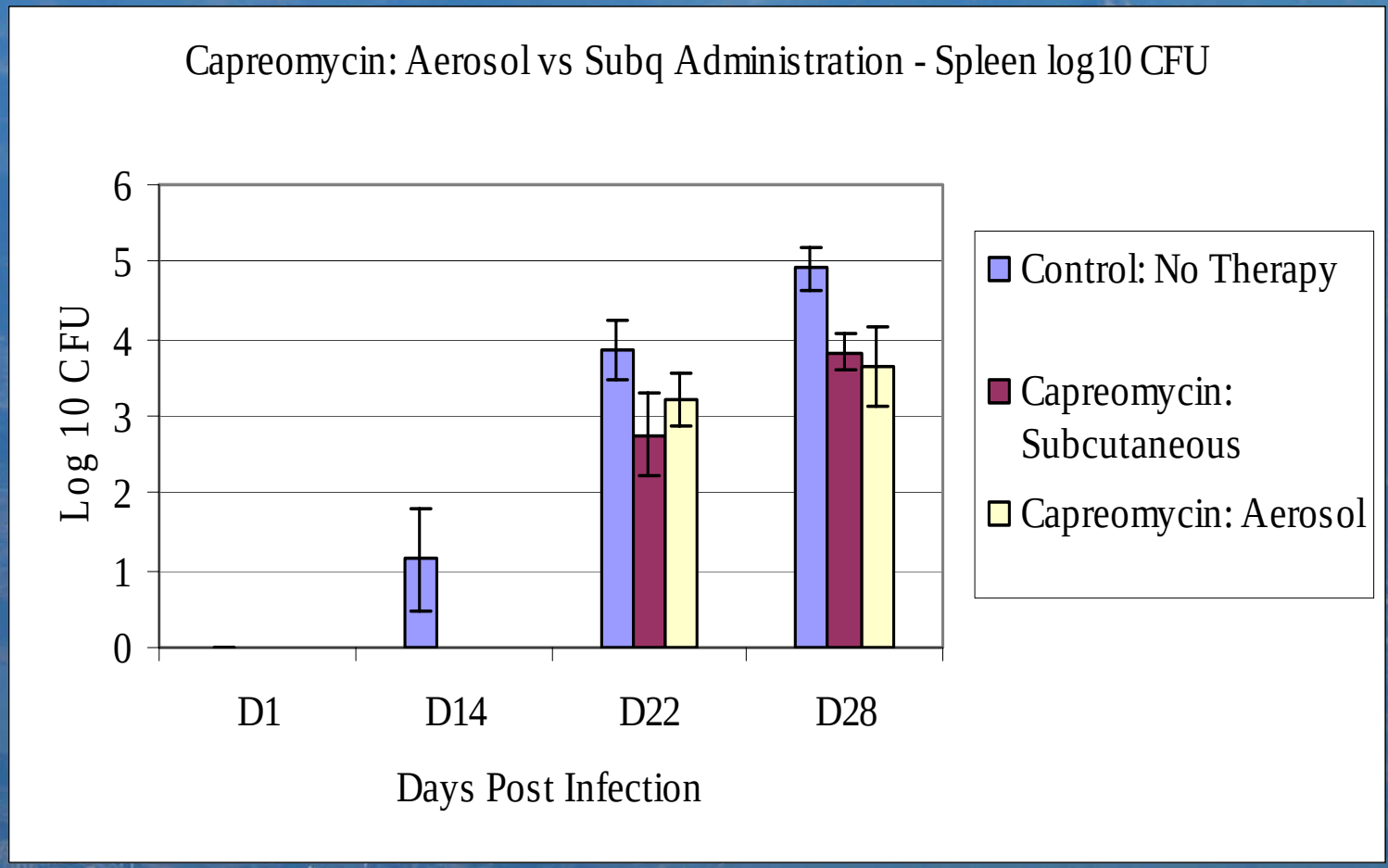
Efficacy of Aerosol Liposomal Rifampicin + Isoniazid : Log10 Lung CFU



Aerosol Capreomycin Efficacy: Lung CFU

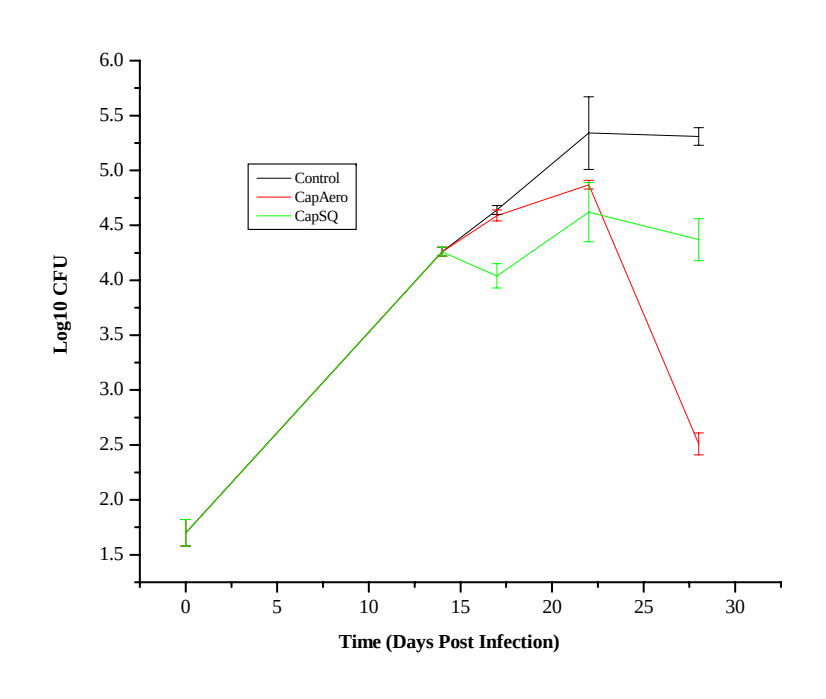


Aerosol Capreomycin Efficacy: Spleen CFU

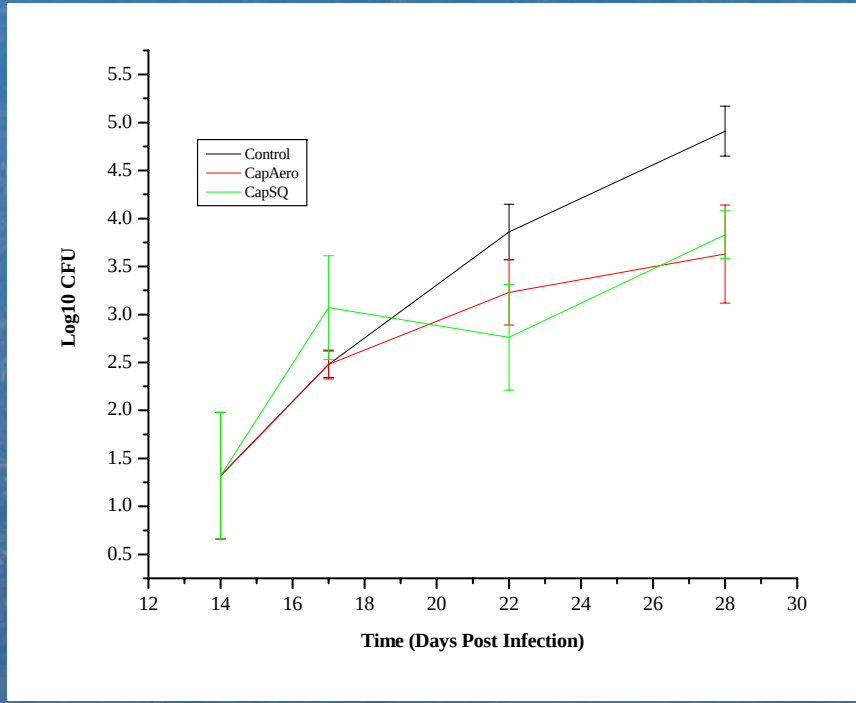


Aerosol Capreomycin: Early Treatment Model (2 weeks post infection)

Lung

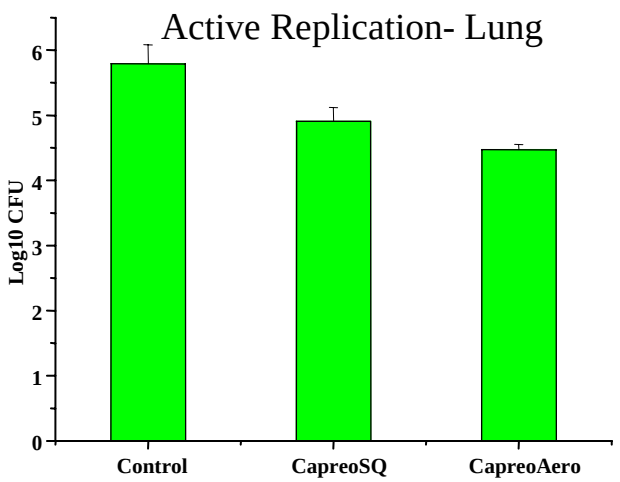


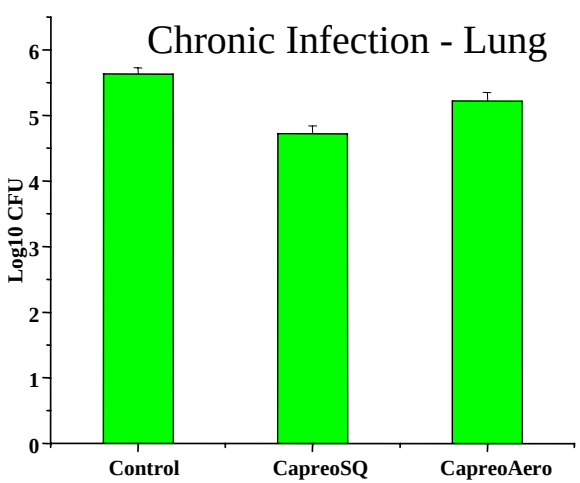
Spleen

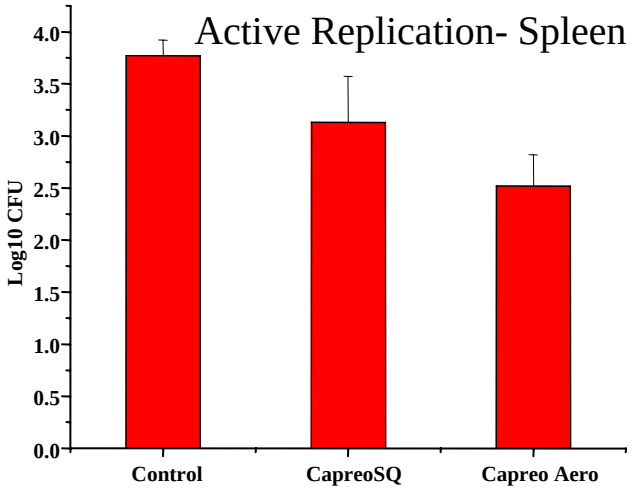


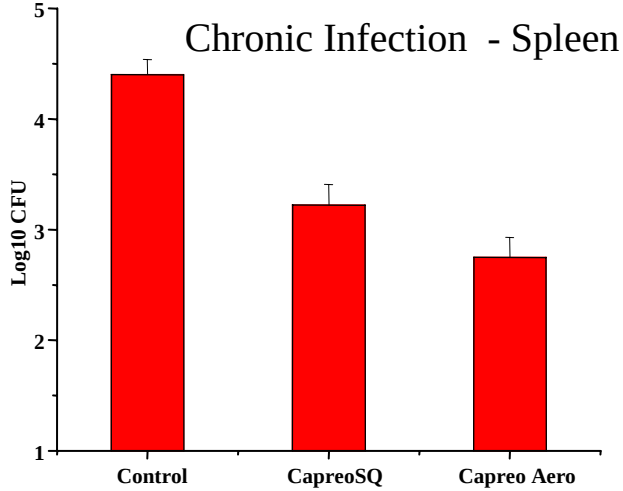
Efficacy of Aerosol Capreomycin

2 weeks and 13 weeks post infection



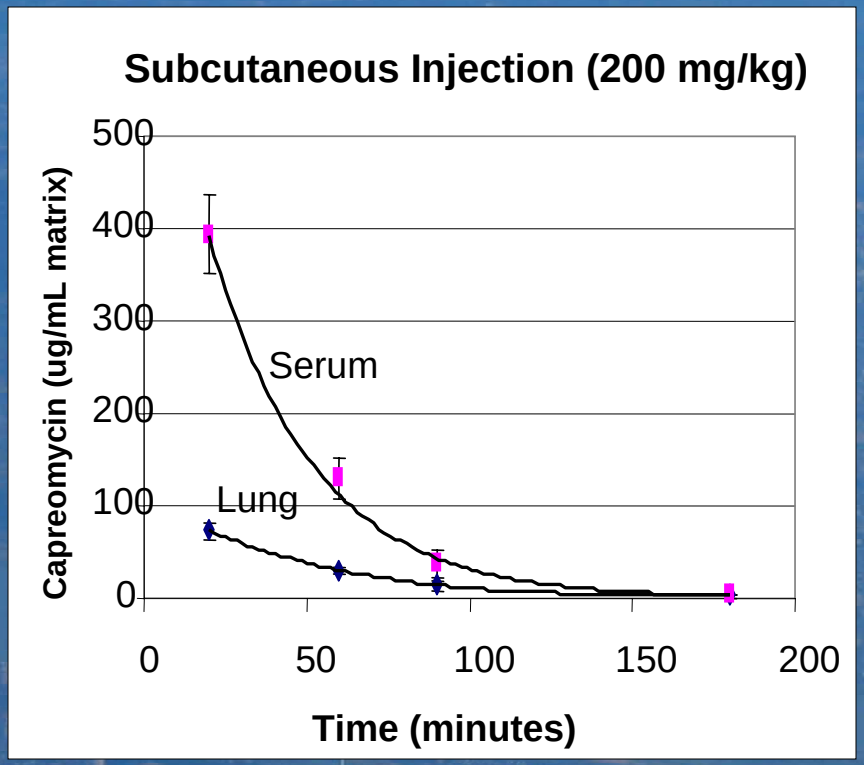


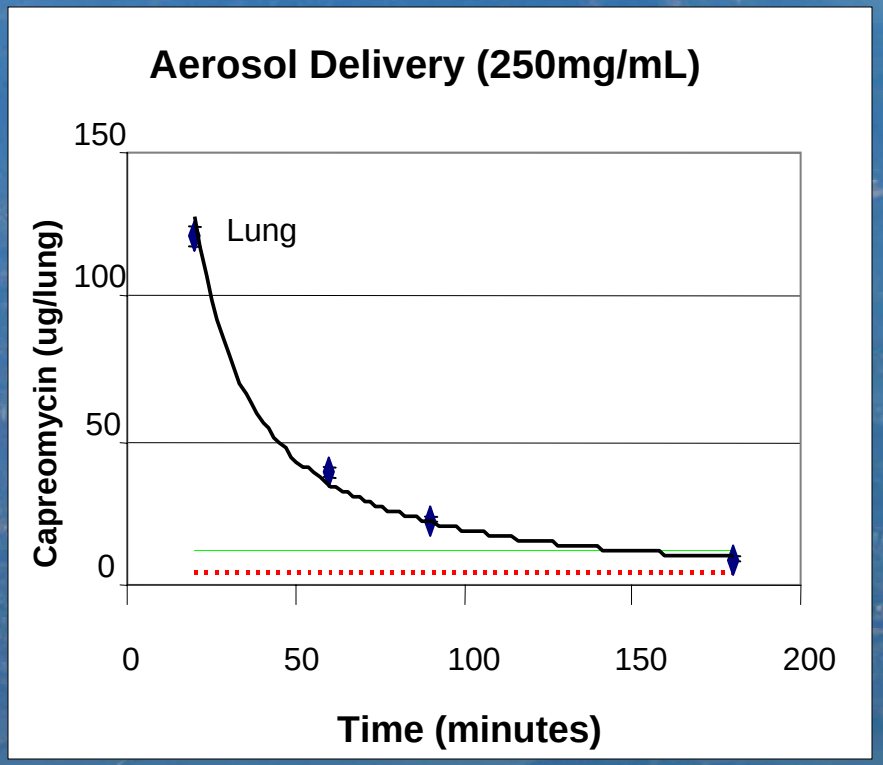




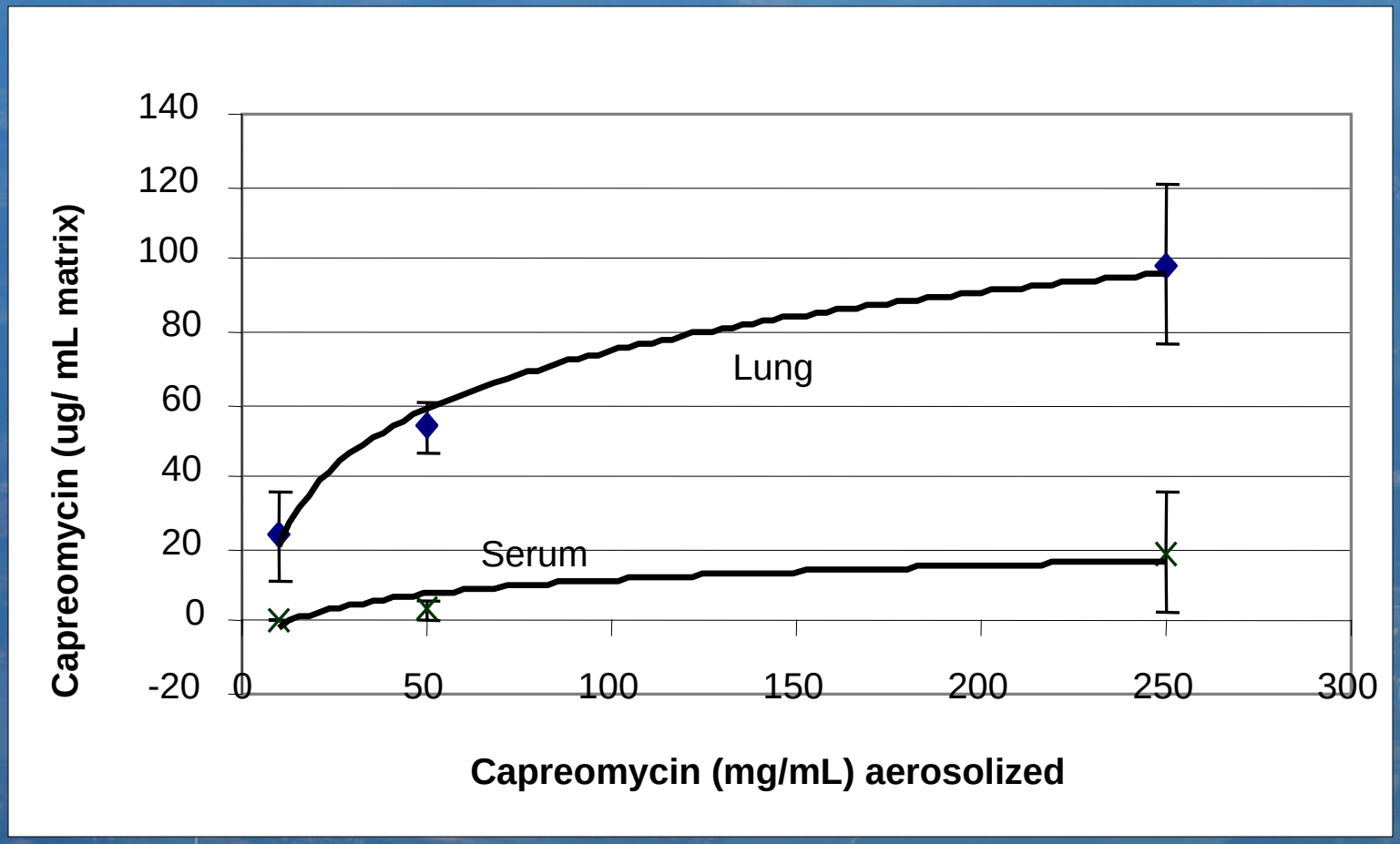
Pharmacokinetics of Capreomycin C57/BL6 after a single exposure

Aerosol delivered for 30 min at 3 lpm using a BANG nebulizer





Lung vs Serum Capreomycin Concentration after Aerosol Exposure (14 days)



Aerosol therapy review:

- 1. With attempts at PK matching, equivalence obtained with Rif/INH.
- 2. Capreomycin *may* have some unique properties - Spleen CFU was without significant reduction while lung CFU 2 log difference - implications for second line agents/injectables.
- 3. Method for Capreomycin detection from serum and tissue developed

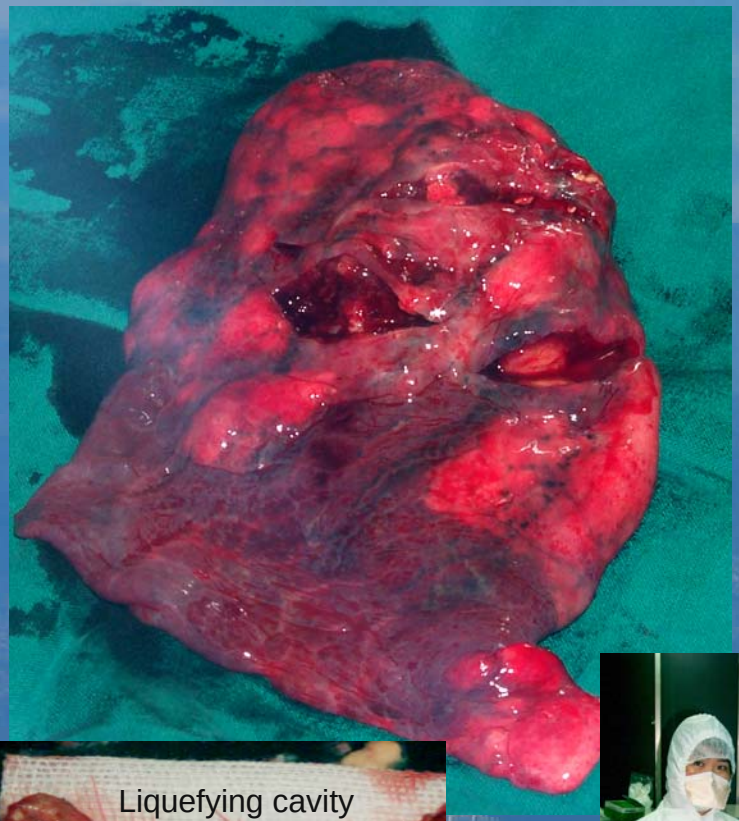
Goal: Develop a clinical trials site for new antituberculars in an MDR setting

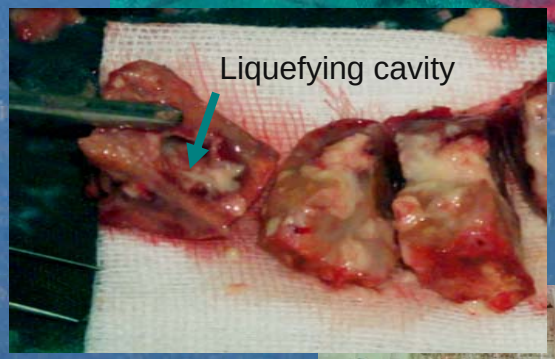
Clinical Research Objectives:

Phase I/II evaluation of aerosol capreomycin

EBA protocol development and validation

Evaluation of drug cavity penetration





Clinical Projects –– South Korea

Masan National Tuberculosis Hospital, Busan, South Korea

National referral center for TB treatment failures
1000 inpatients/yr
50% of inpatients are MDR
5000 outpatients/yr
20-50 lobectomy surgeries/yr
Very low HIV+
KIH donated former 4000 sq ft lab adjacent to MNTH

Division of labor in capreomycin aerosol development

NIH:

- Mouse PK/PD/Tox
- Additional Efficacy studies
- Pre-IND FDA: Orphan Grant

Lilly:

- Dog tox protocol in development with Dr Wolff

Masan NTH:

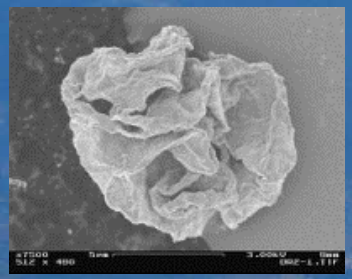
- IRB Composed
- Phase I protocol pending

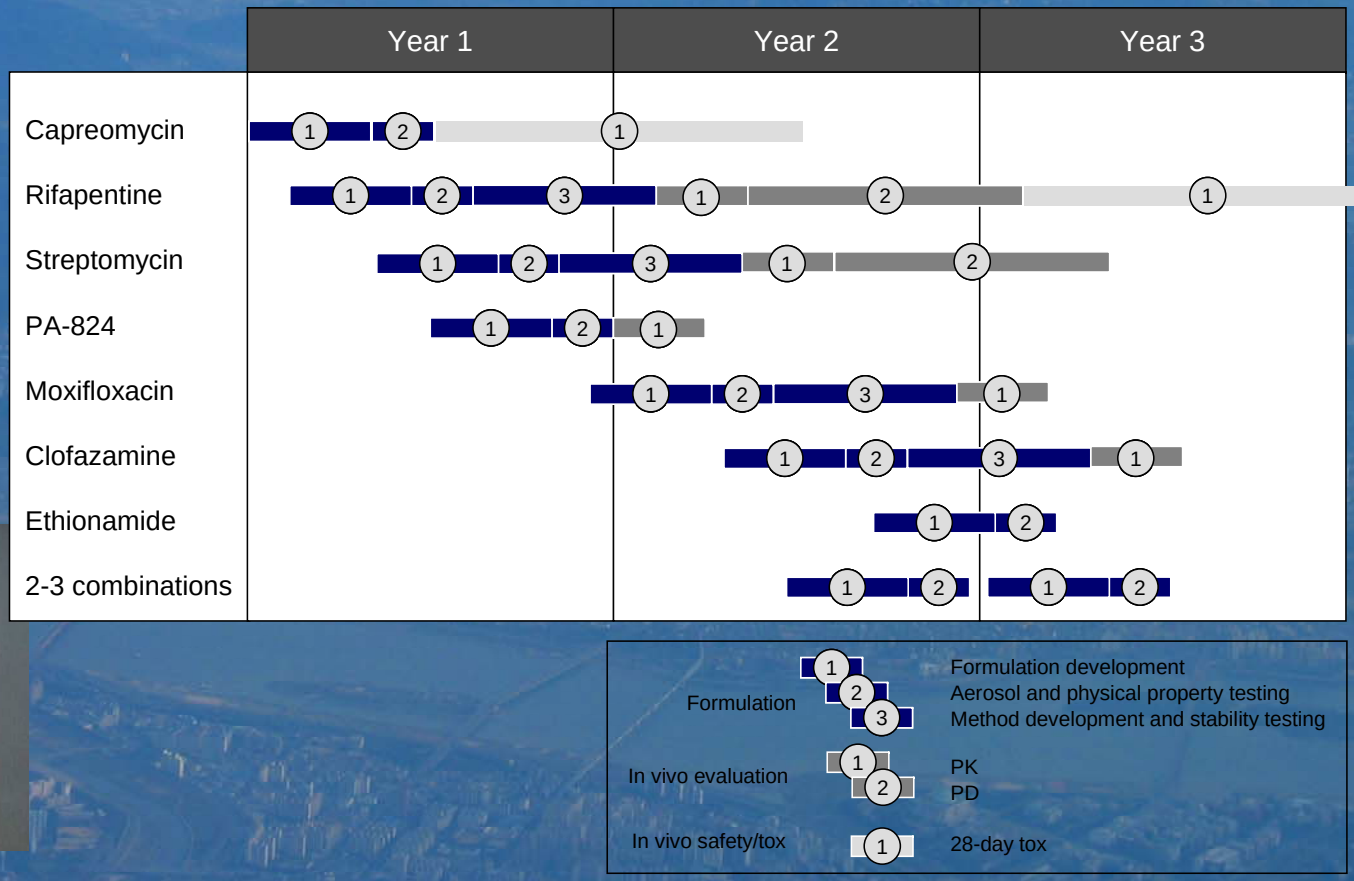
Additional aerosol delivery systems

David Edwards, Harvard

Agents are spray dried using a NIRO Atomizer Portable spray-drier (Columbus, MD)

Formulation with ethanol/water solutions and other solvents, excipients, amino acids, and common lung surfactants (e.g., DPPC) to make large porous particles LLP





Clifton Barry's TRS Members and Collaborators

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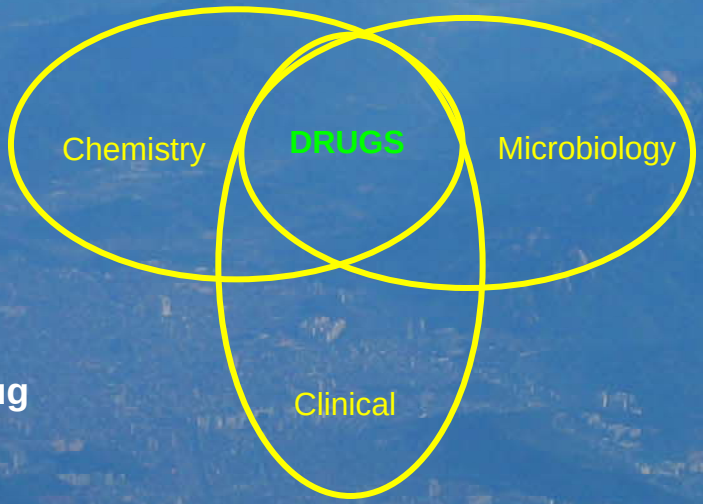
L Zhang
M Goodwin
QA Nguyen



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