Alpha-crystallin: A Marker of Latent TB Infection

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Institute Of Medicine Report

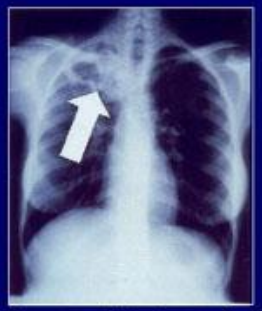
Ending Neglect – The elimination of Tuberculosis in the United States (2002)

- "the **single most valuable diagnostic test for use in the United States** would be one that could predict which latently infected individuals are most at risk of developing active tuberculosis." As further stated in the report "**.....because so many of the cases in the United States are not the result of recent transmission of tuberculosis, but rather are the result of reactivation of latent infection, the greatest needs in the United States are new diagnostic tools for the more accurate identification of individuals who are truly infected and who are at risk of developing tuberculosis.**"

TB Diagnostics

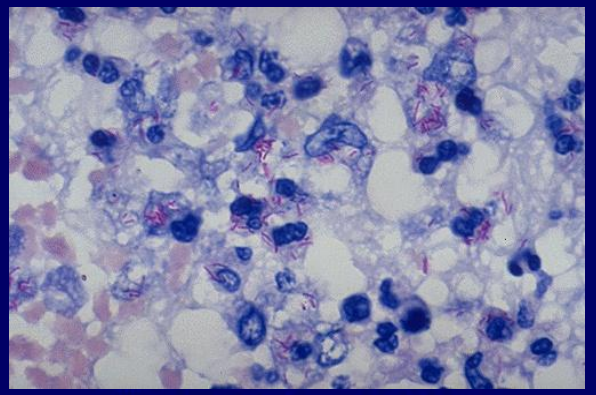
Chest Radiograph

- Abnormalities often seen in apical or posterior segments of upper lobe or superior segments of lower lobe
- May have unusual appearance in HIV-positive persons
- Cannot confirm diagnosis of TB



Arrow points to cavity in patient's right upper lobe.

AFB smear



AFB (shown in red) are tubercle bacilli

Culture

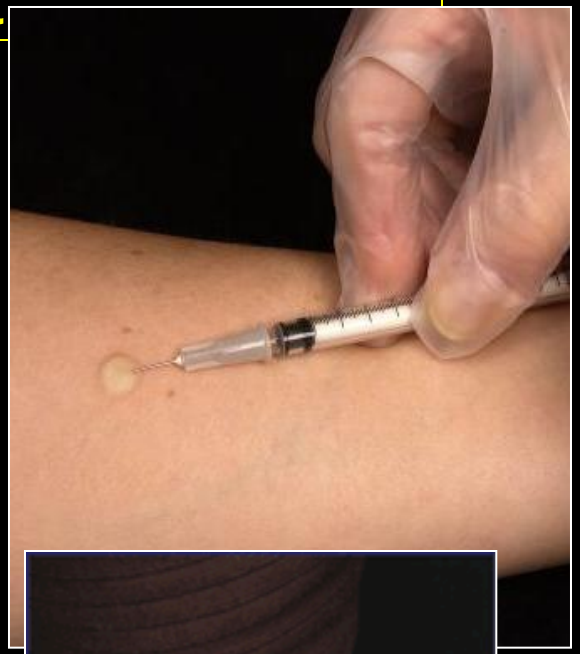
- Results in 4 to 14 days when liquid medium systems used

Colonies of *M. tuberculosis* growing on media

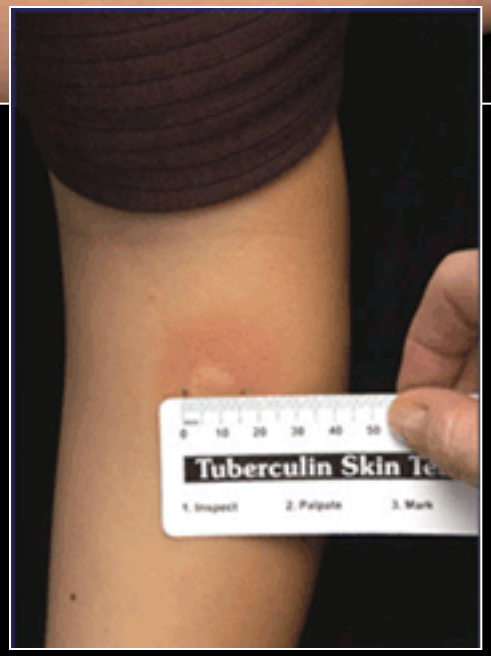
TB Diagnostics

Tuberculin Skin Test

- Inject 0.1 mL PPD intradermally
- Should produce wheal of 6–10 mm



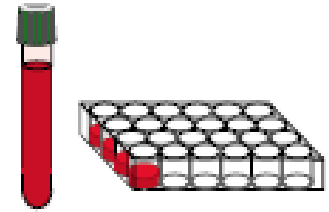
- Read 48–72 hrs after placement
  - If HCW returns after >72 hrs, place and read another TST*
  - Do not let HCWs read their own results
- Find and measure induration
  - Measure diameter of induration across the arm
  - Do not measure redness



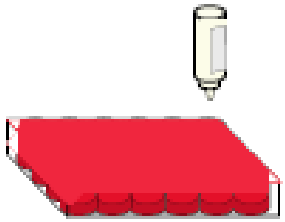
TB Diagnostics

QuantiFERON®-TB Gold Test

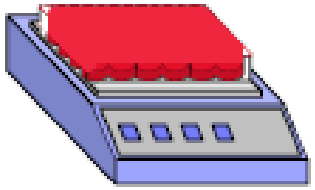
Stage One – Blood Stimulation and Harvesting



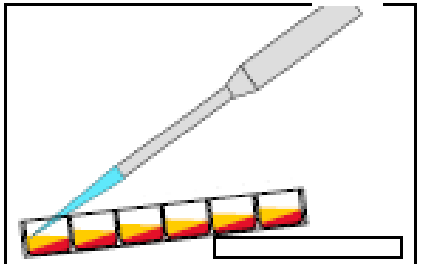
Dispense 1 mL of subject's heparinized whole blood into 4 wells of a 24-well culture plate.



Add 3 drops of the appropriate stimulating antigen to each well.

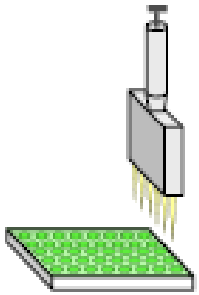


Shake covered plate for 1-2 min. Incubate for 16-24 hrs at 37°C (humidified).

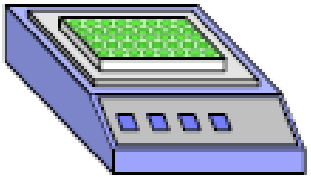


Harvest at least 200 µL plasma from each well. Store in racked microtubes or uncoated microplates.

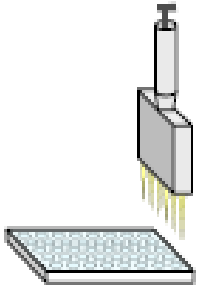
Stage Two – Human IFN-γ ELISA



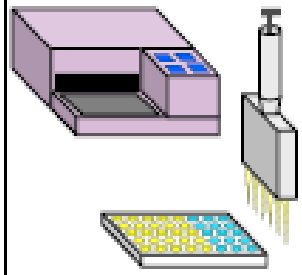
Add 50 µL of conjugate solution to each well. Add 50 µL of plasma or standard.



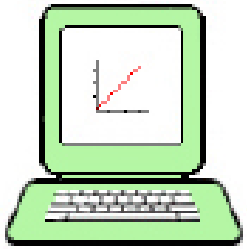
Shake covered plate for 1 min. Incubate for 120 minutes at Room Temperature.



Wash plate ≥ 6 times. Add 100 µL of substrate. Incubate 30 min. at Room Temperature.



Add 50 µL of stop solution. Read absorbance within 5 min at 450nm (620-650nm ref).



Calculate Results using standard analysis programs (QFT-Gold Analysis Software available soon).

TB Diagnostics

Chembio lateral-flow serological tests

- non-human primates (PrimaTB STAT-PAK™)
- white tail deer, reindeer, and elk (CervidTB STAT-PAK™)
- cattle (BovidTB STAT-PAK™)
- badgers (BrockTB STAT-PAK™)
- camels, llamas, and alpacas (CamelidTB STAT-PAK™)
- elephants (ElephantTB STAT-PAK™)

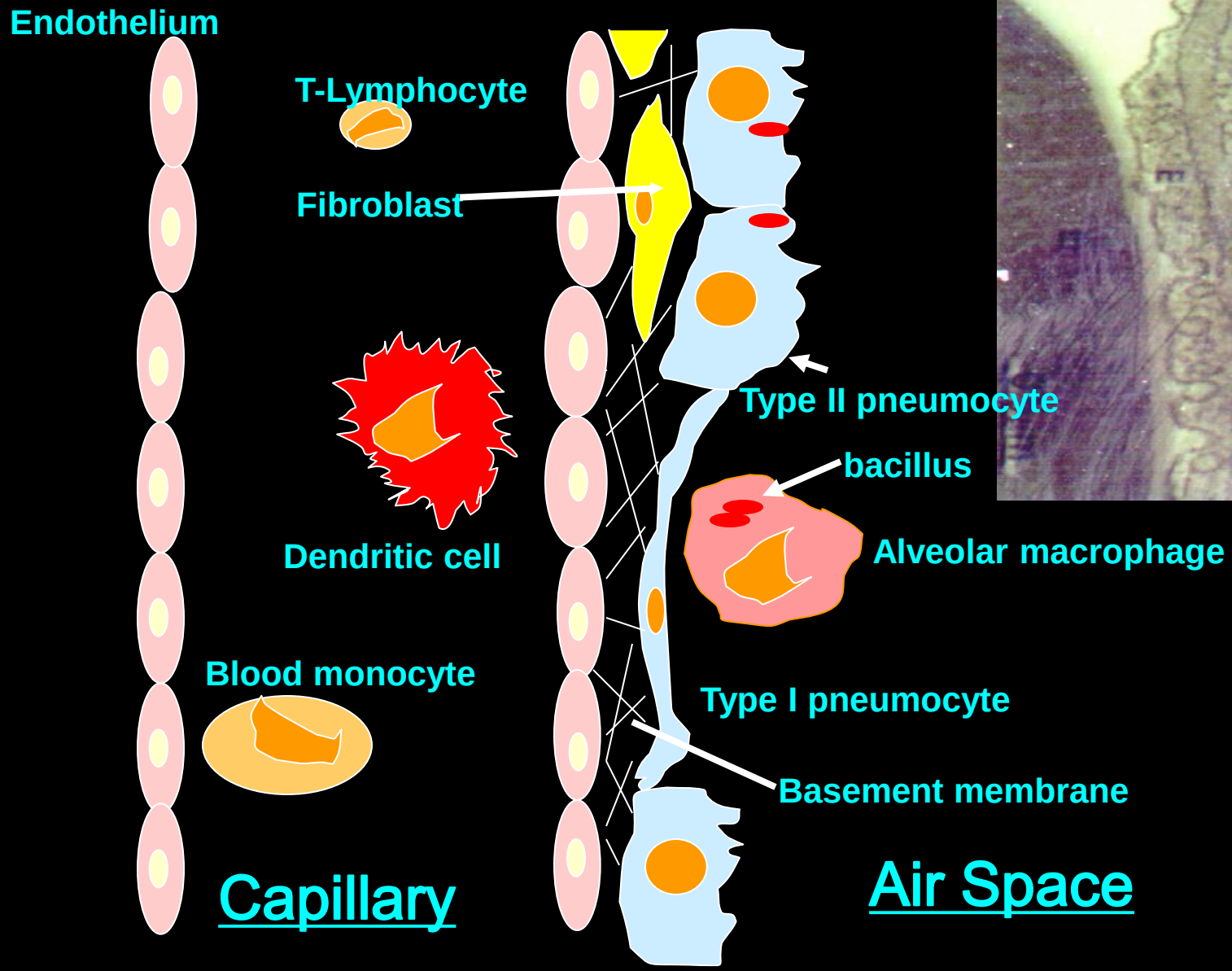
- antibody detection assays that employ cocktails of recombinant antigens from *M. bovis* and *M. tuberculosis*
- The tests can use serum, plasma, or whole blood samples and yield a result result in 20 minutes

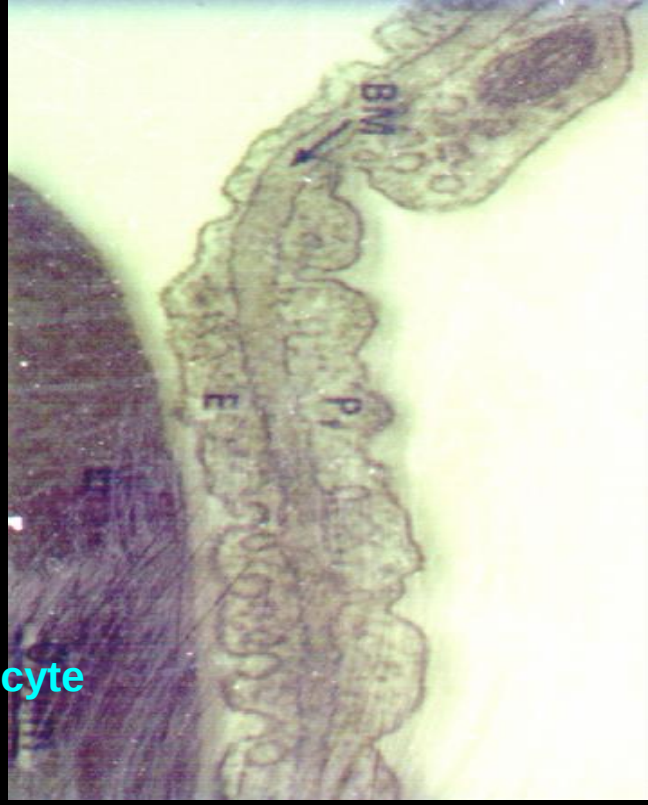
Many of these tests and others have been used to detect both LTBI and active TB, but cannot discriminate

Phase 1: Alternative Approach

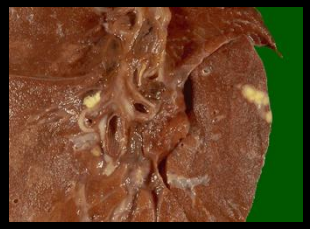
- High specificity in a test for latent TB could be achieved by detecting the presence of a specific latency-associated bacterial antigen(s), rather than measuring the host immune response
- Look in the granuloma…

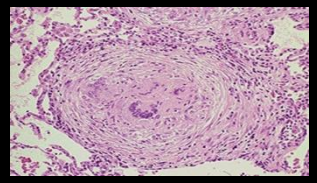
Early infection

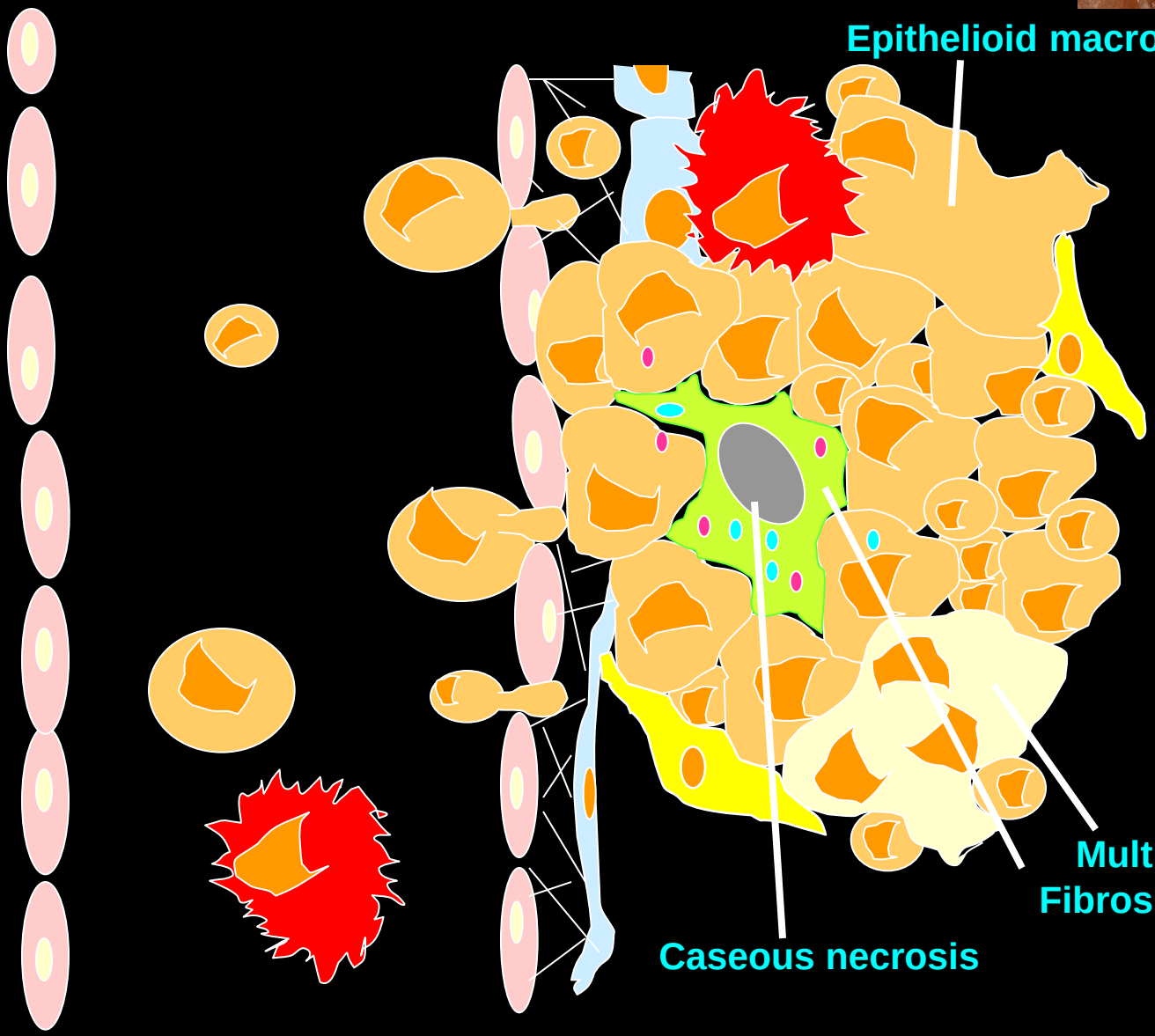




Extravasation and Granuloma Formation







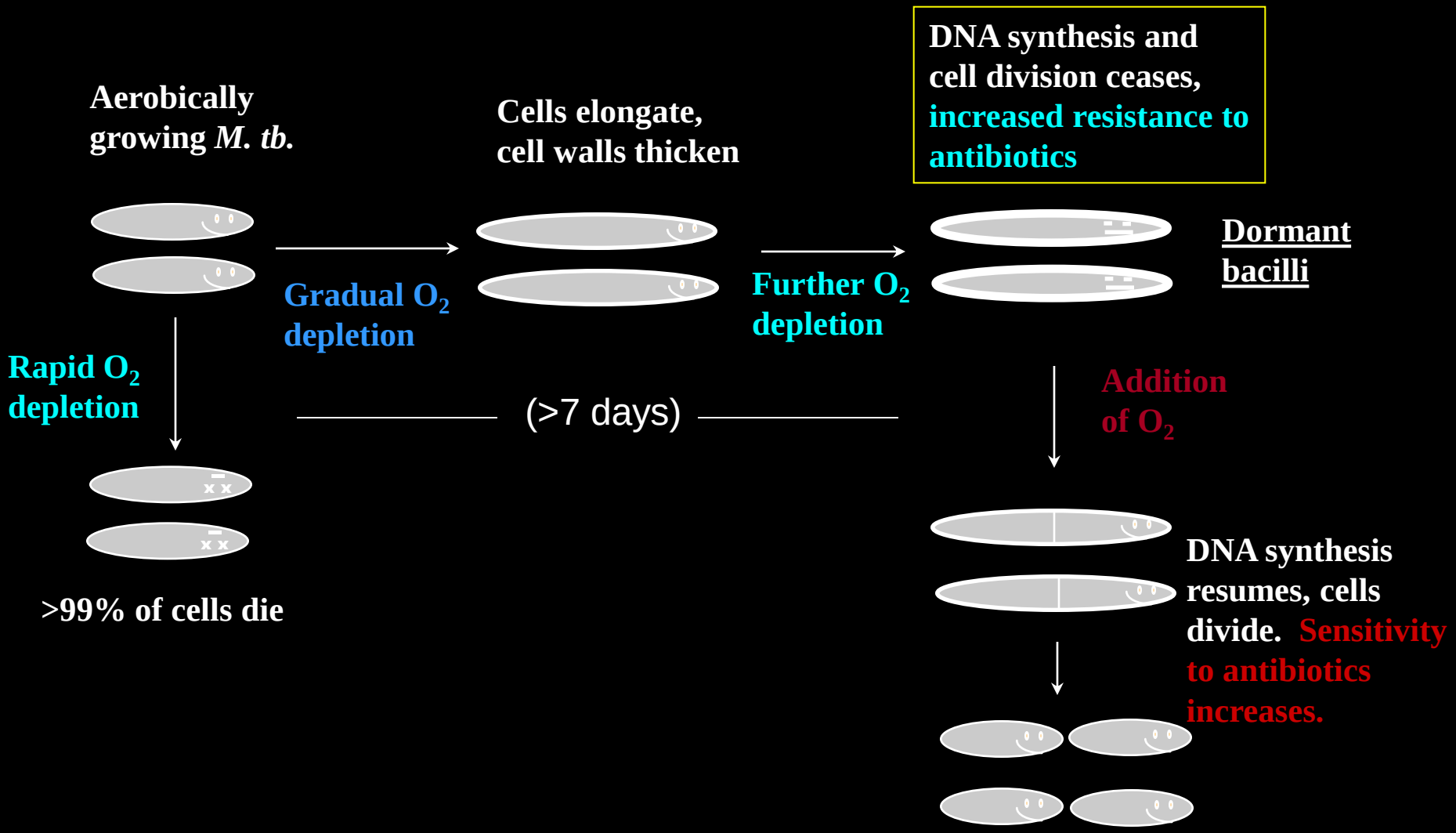
Suspected/Confirmed Conditions within TB Granuloma

- Within granuloma: low O_2, pH and nutrient levels; high levels of lipids
- Bacilli are non or slowly replicating though metabolically active

Models that may mimic these conditions

-- hypoxic chamber
-- *in vitro* granuloma
-- *in vivo* lung granuloma

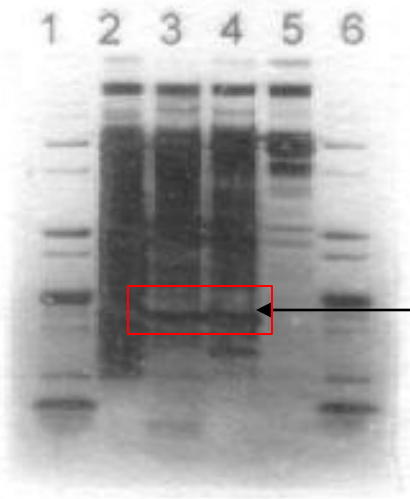
M. tuberculosis bacilli can enter a dormant state *in vitro*



Total protein analysis

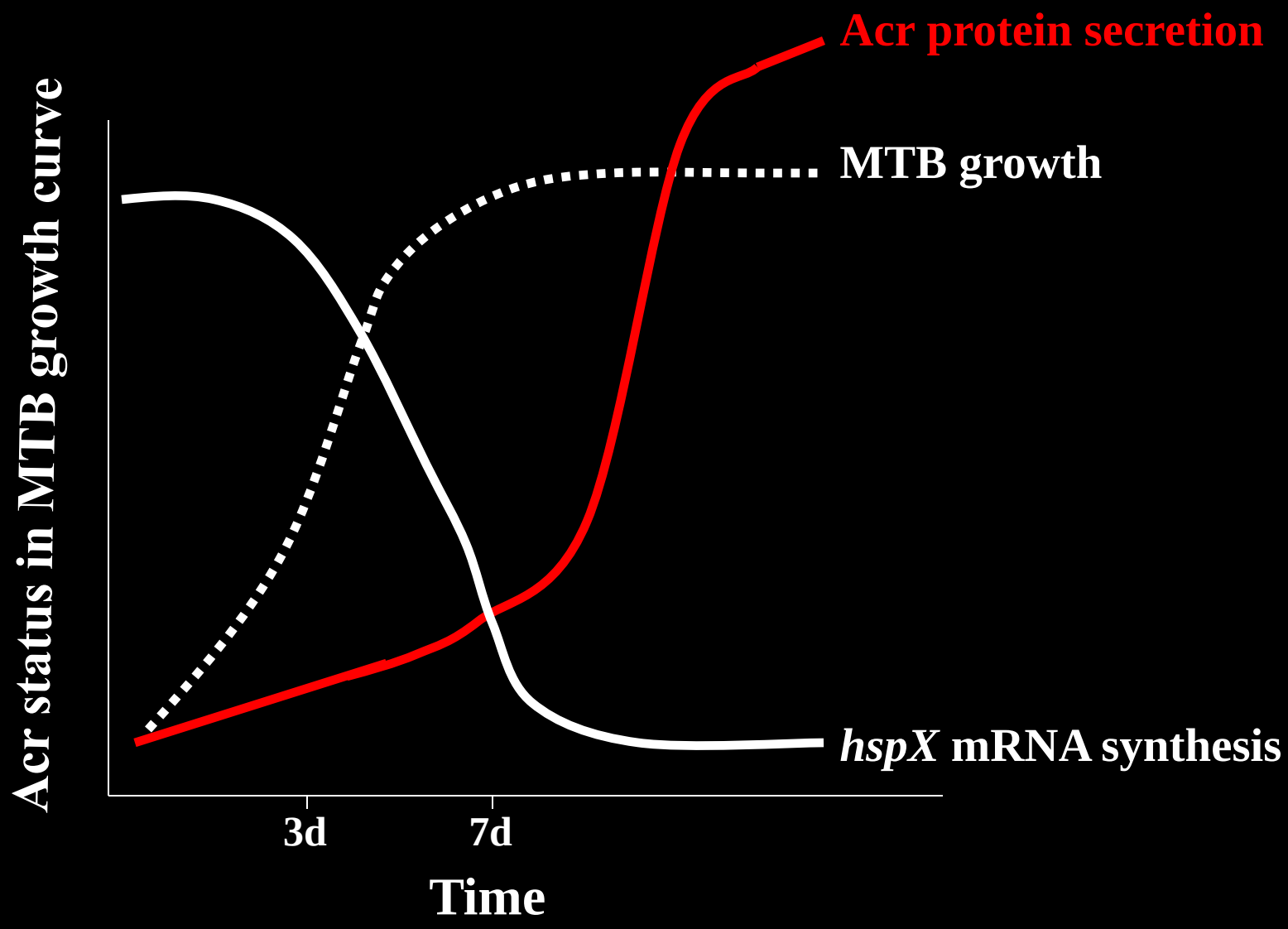
Mtb bacilli from various *in vitro* growth stages

Lane
1 Marker
2 Aerobic
5 days (mid-log)
3 Latent
11 months (NRP2)
4 Anaerobic
7 days (NRP1)
5 Reactivated
30 hours
6 Marker



17Kda (Acr)

Acr regulation

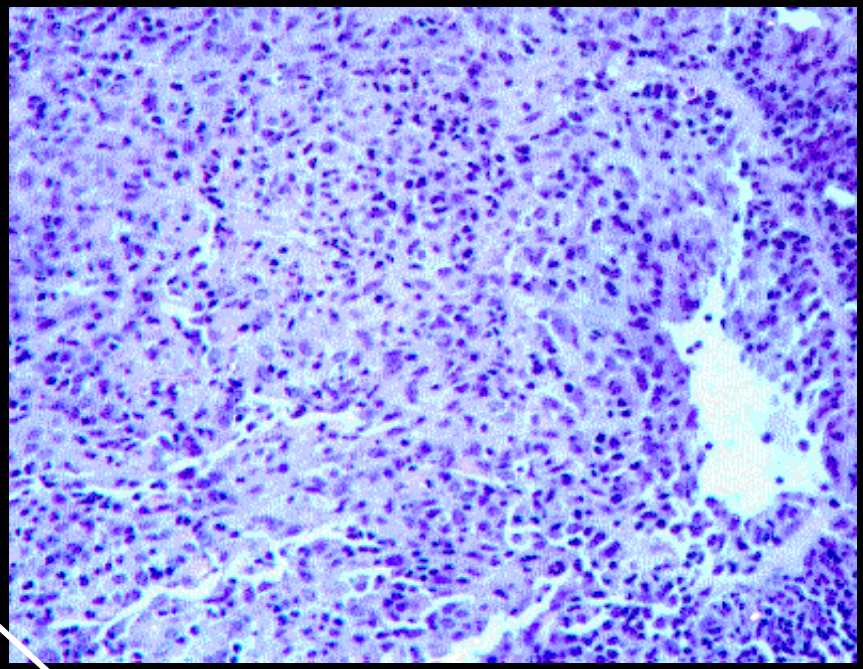


Alpha-Crystallin homolog (Acr)

- encoded by *hspX/Rv2031c* – first gene in four gene operon
- small 144 amino acid protein
- DosR regulated
- possesses heat shock and chaperonin characteristics
- undetectable in log-phase cultures
- predominantly produced in stationary-phase
- correlates with cell wall thickening
- localized with in cell wall
- expression induced during *in vitro* infection of macrophages
- mutant strain ΔhspX produces hypervirulent phenotype in aerosol mouse infections
- function unknown

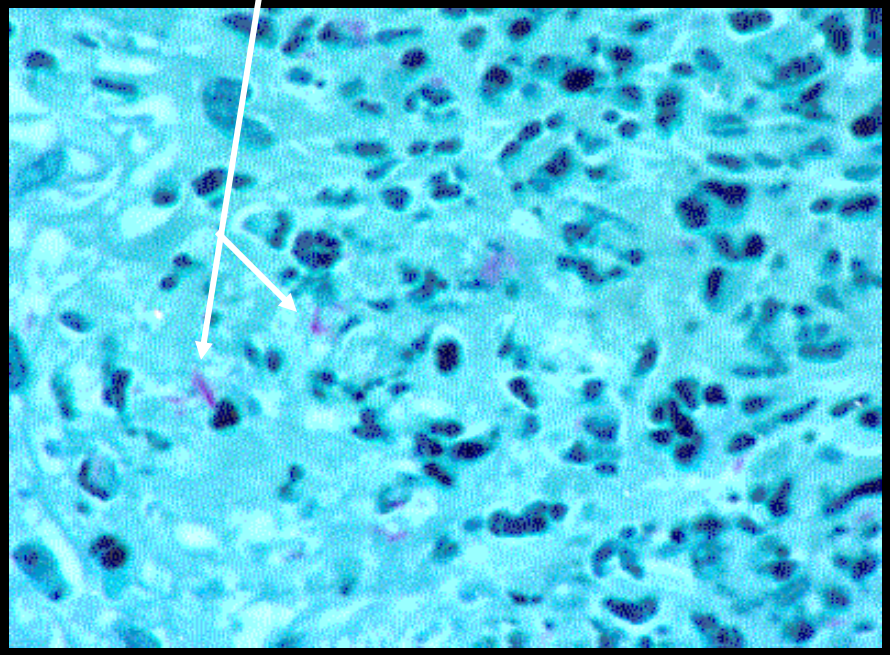
H&E stain

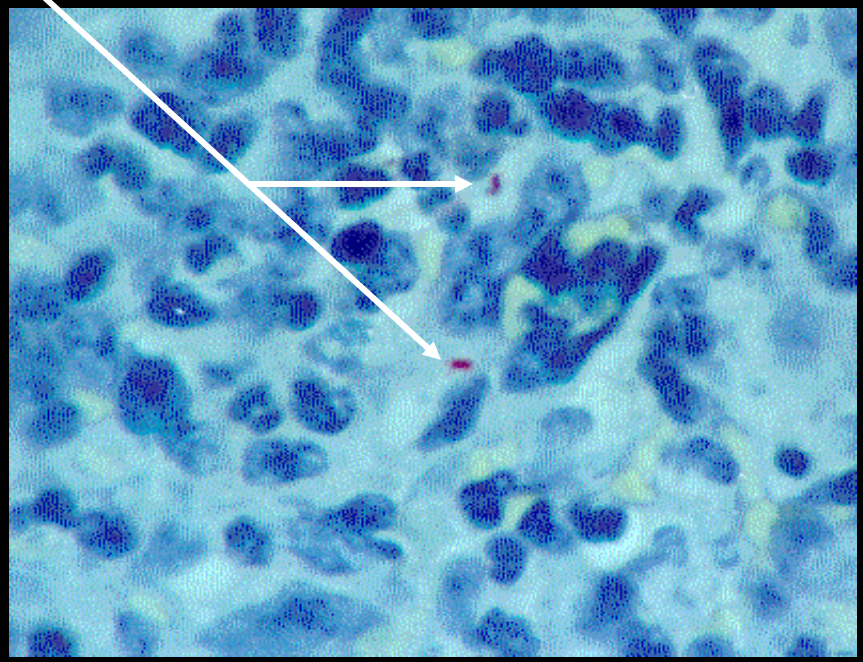
Aerosol-infected Guinea Pig lung granuloma- 3wk.

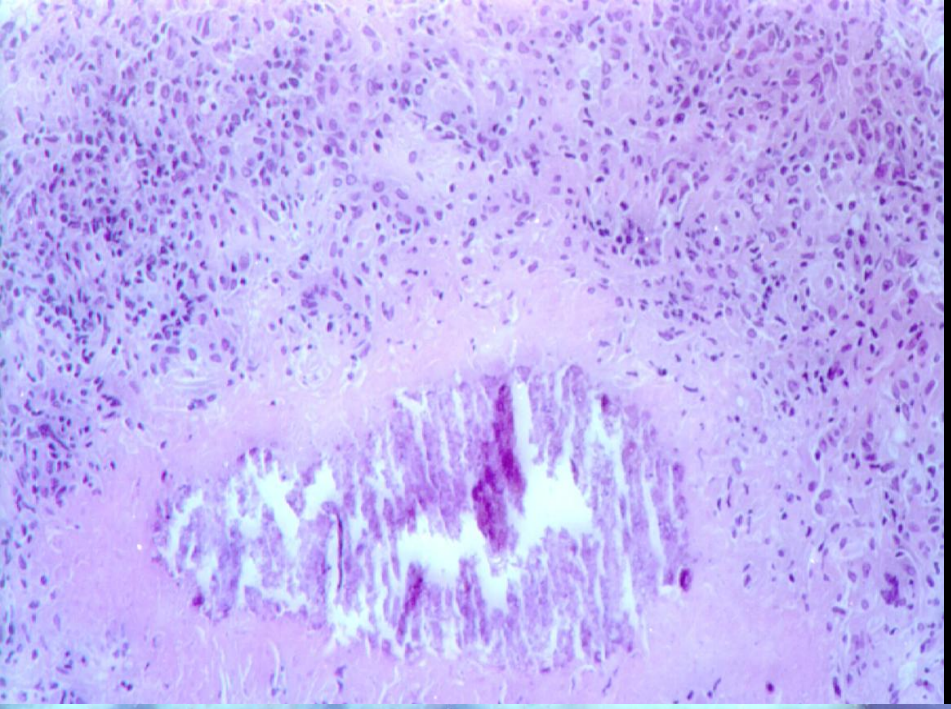


αAcr IHC stain

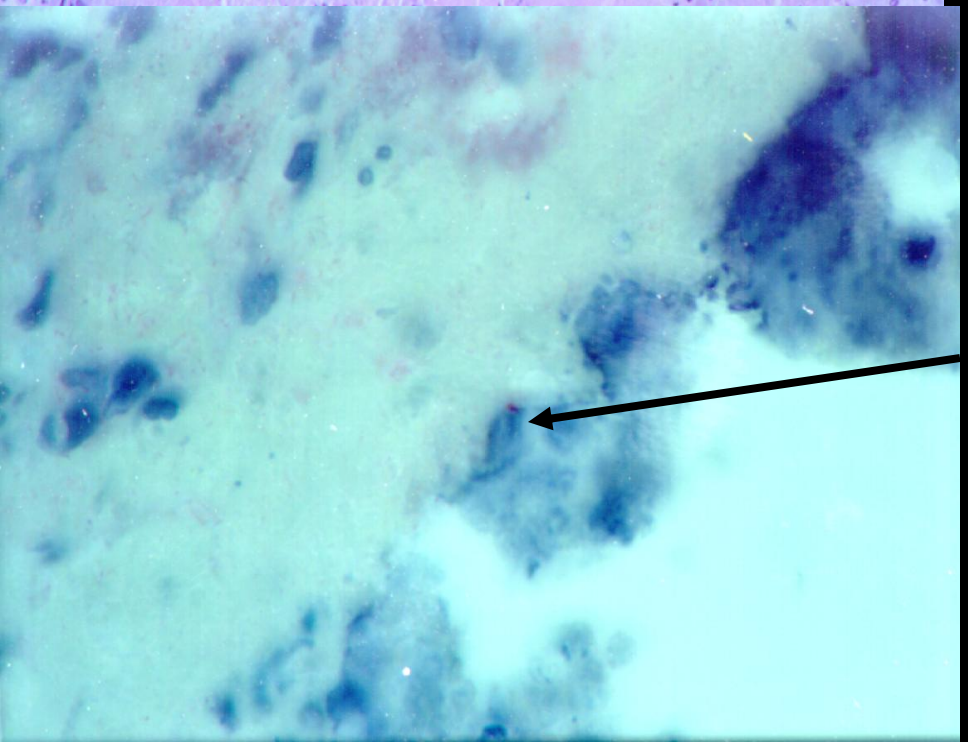
Acid fast stain







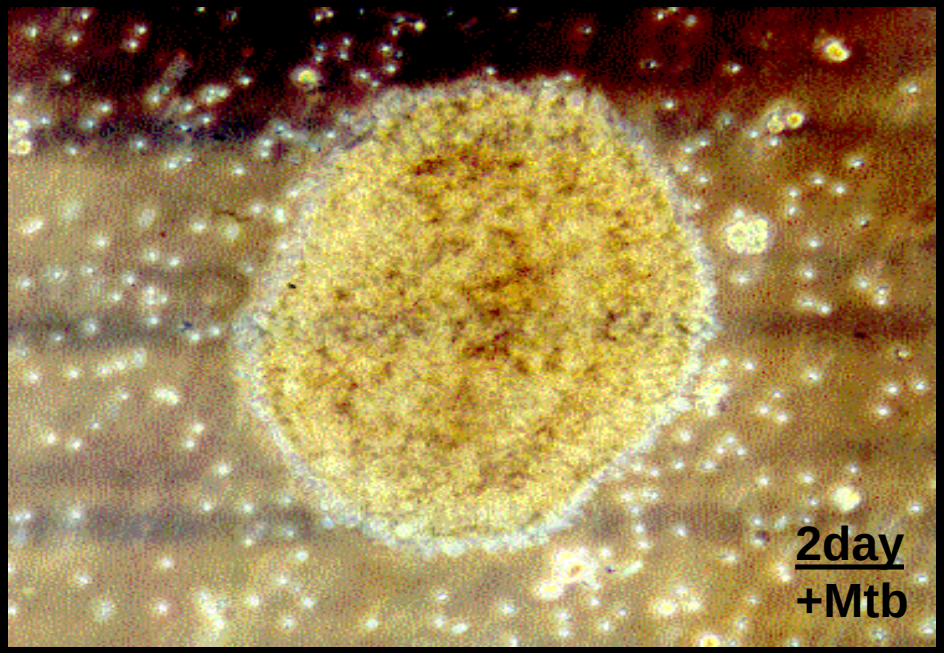
Aerosol-infected rabbit lung granuloma-10wk.

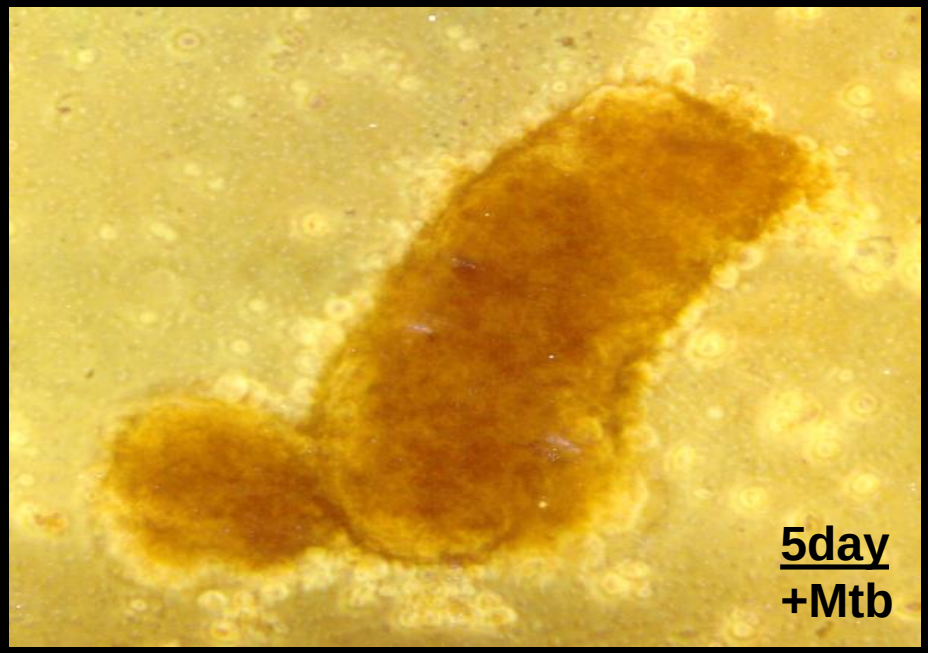


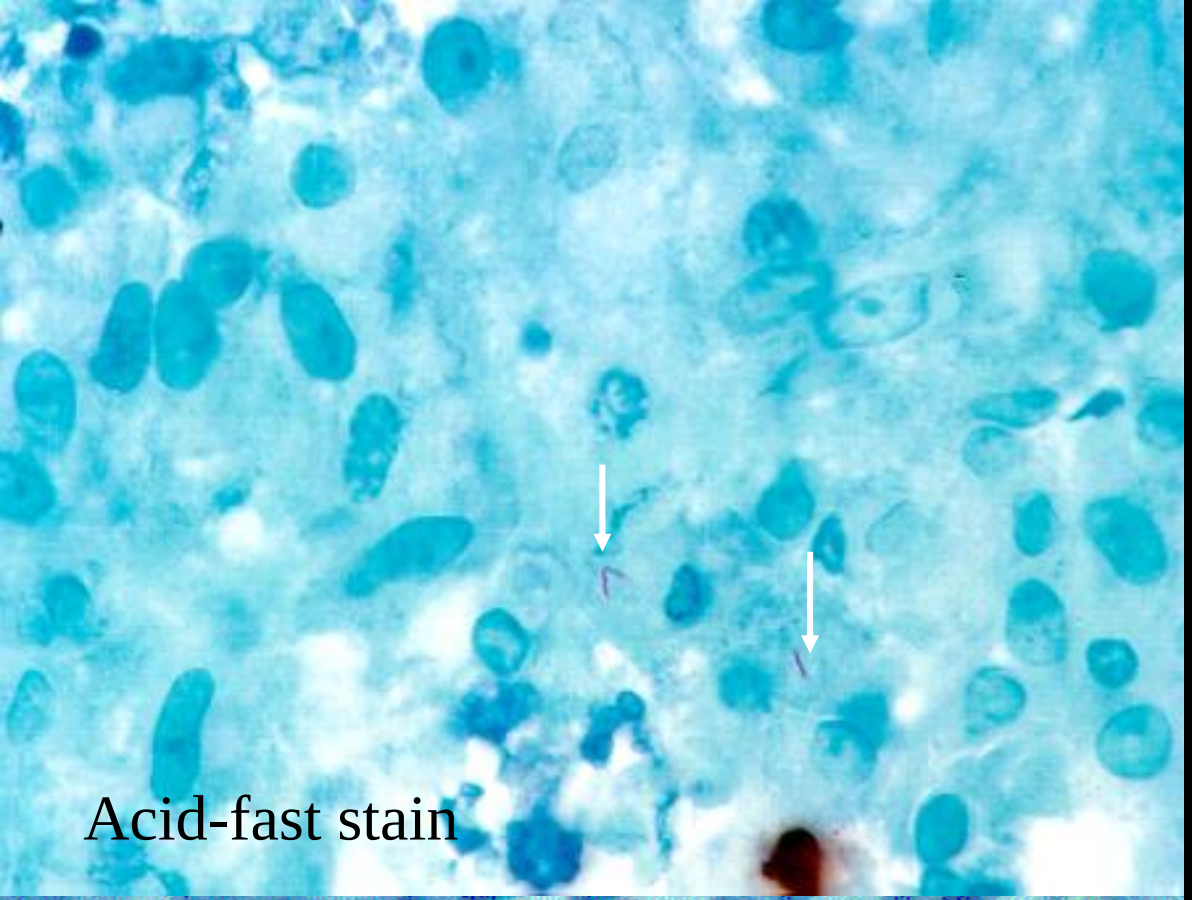
αAcr IHC stain

In vitro granuloma

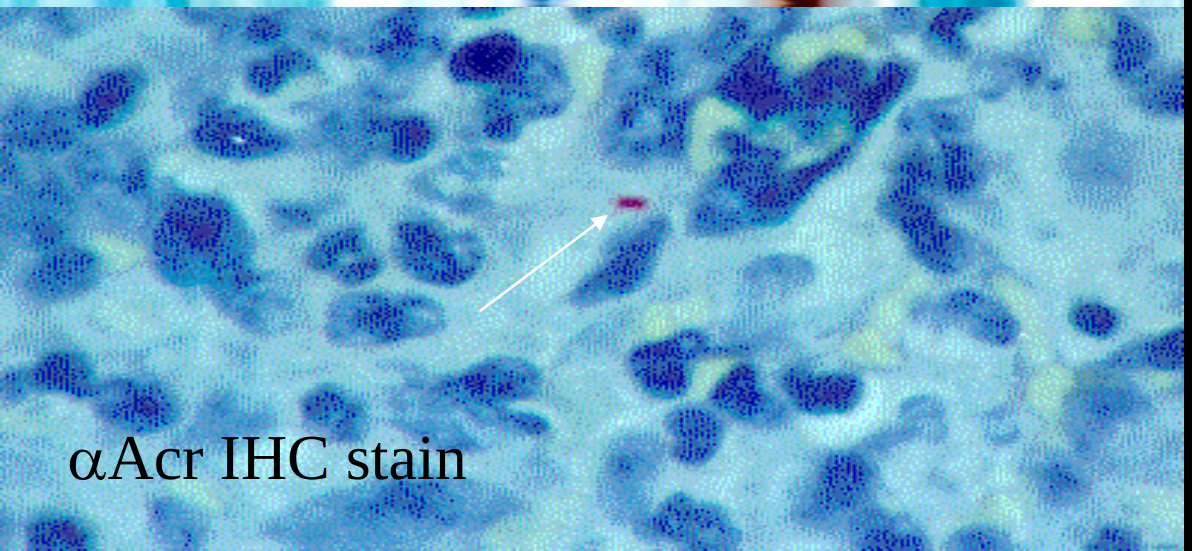
(Birkness, et al., Microbiol. 2006)







Acid-fast stain

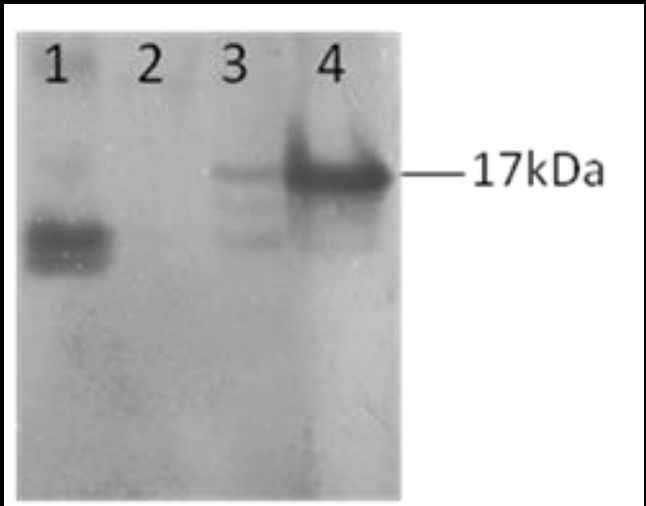


αAcr IHC stain

***In vitro* Granuloma**
5-day

Immunoblot analysis of *Mtb* culture supernatants

probed with anti-Acr rabbit polyclonal antiserum

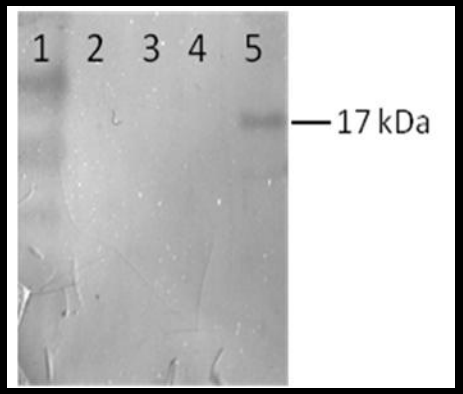


Lane 1, 12-month hypoxic culture
Lane 2, host cell control lysate
Lane 3, 9-day in vitro granuloma
Lane 4, 14-day stationary phase culture

Phase I: Preliminary Acr Serum Diagnostic
probed with anti-Acr monoclonal antibodies (2 of 30)

Test material	IT-4-probed Western blot
PBS + 1 pM rAcr	+
NHS + 10 pM rAcr	+
NHS control	-
Normal guinea pig sera	-
Sera from 3-week infected guinea pigs	5/5
Group 1 human test sera	4/5
Group 2 human test sera	3/5
Group 3 human test sera	0/5

- Group 1, individuals with well-documented active disease, and in the early stages of chemotherapy
- Group 2, possibly latently-infected individuals, positive on the tuberculin skin test, but show no evidence of active infection, but in close contact with active TB cases
- Group 3, healthy, skin test negative individuals with no known contact with TB cases



Lane 1, MW marker
Lane 2, 10 femtomolar (fM) rAcr
Lane 3, 100 fM rAcr
Lane 4, 1 picomolar (pM) rAcr
Lane 5, 10 pM rAcr

Phase II: Clinical Screen

- In the process of screening sera by ELISA for Acr
  - 35 human patients with active/acute tuberculosis,
  - 31 PPD-positive, asymptomatic close contacts
  - 50 PPD-negative controls

Future Directions

- Additional *Mtb* latency-specific proteins to be included in the assay
- Continue to screen additional antibodies and methodologies to enhance sensitivity
- Screen urine samples
- Collect samples from more “complicated” populations
  - e.g. HIV+, BCG-vaccinated
- Identify latent infections in animal patients
- Determine efficacy of latency therapies
  - Animals and humans

The Cast

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