

Microbiology of Tb in HIV- infected patients

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Pathogenesis of TB - 1

- **Aerosol travels to alveoli of lungs**
- ***M. tuberculosis* engulfed in alveolar macrophages**
- **if activated (e.g. healthy adult), host may clear bacteria, or at least contain infection**
 - **tuberculin positive**
- **if unactivated (e.g. infant), bacteria survive and replicate in macrophages**
 - **attract more cells (PMN's, T-cells), damage tissue, and form granulomatous tubercle**

Pathogenesis of TB - 2

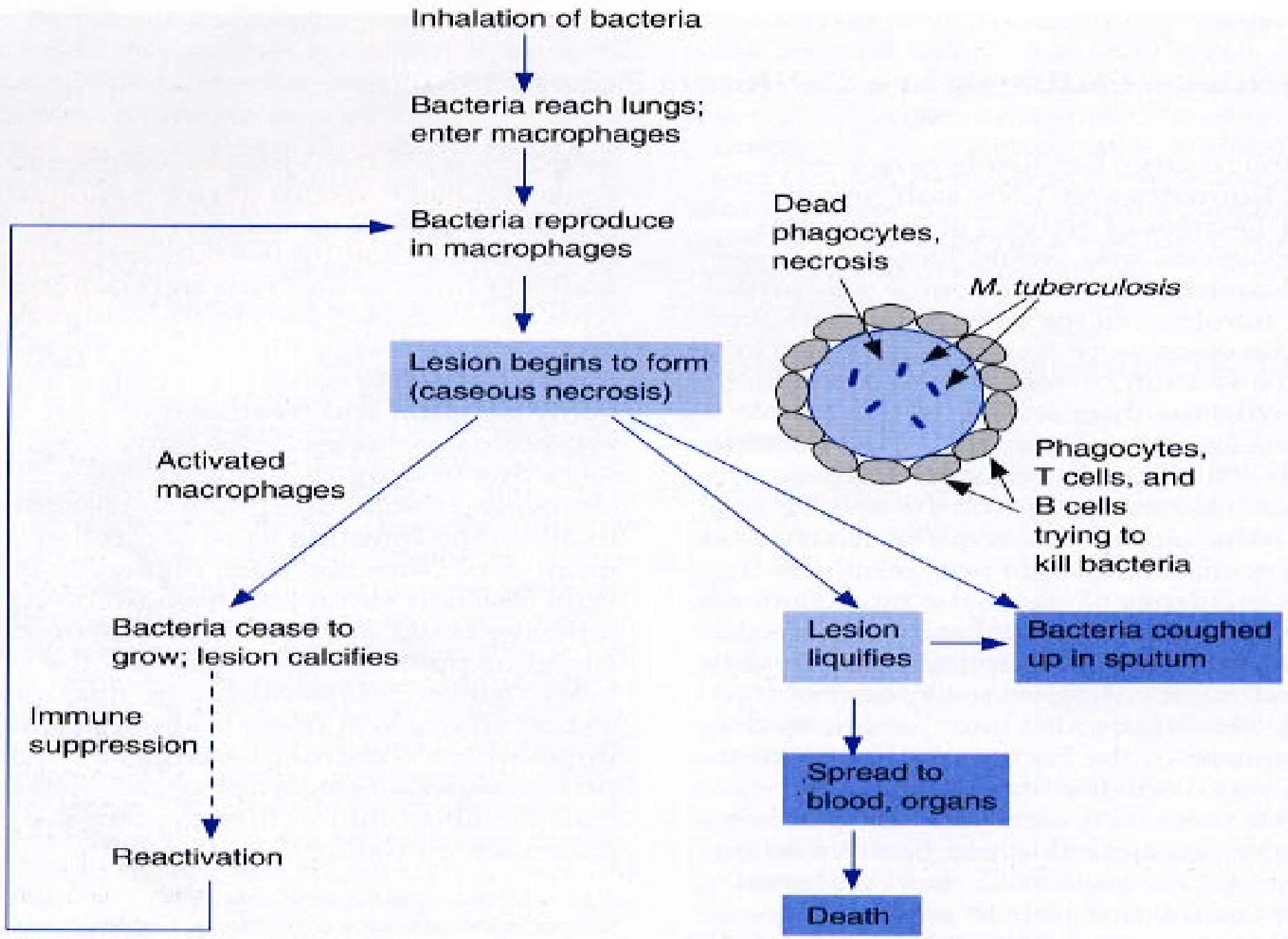
- Tubercle can remain silent (abN X-ray)
- **Granulomatous response may fail to contain bacteria**
 - Immediate lymphatic / hematogenous spread (primary TB)
- **Granulomatous response can result in tissue damage**
 - N.B. TNF - alpha production
- **Granuloma may remain wall off for years to decades, then allow release of viable bacteria: reactivation**

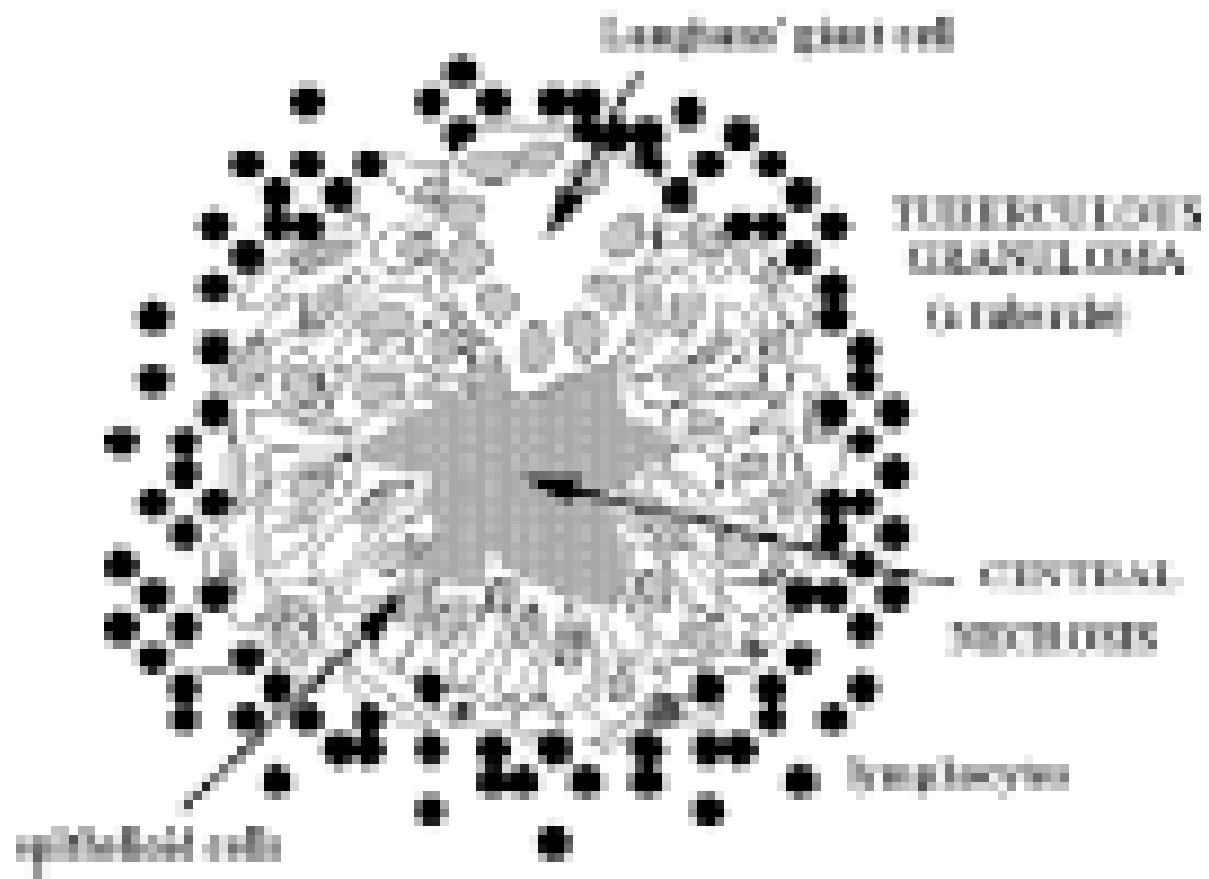
Tumor Necrosis Alpha (TNF alpha)

- TNF alpha increases macrophage ability to kill *M. tuberculosis*
- TNF alpha required for granuloma formation
- Granulomas sequester mycobacteria and prevent uncontrolled dissemination

PATHOLOGY

- Macrophages secrete lytic enzymes which cause tissue necrosis
- Epithelioid cell=highly stimulated macrophage
- Langhans Giant Cell= fused macrophages with multiple nuclei

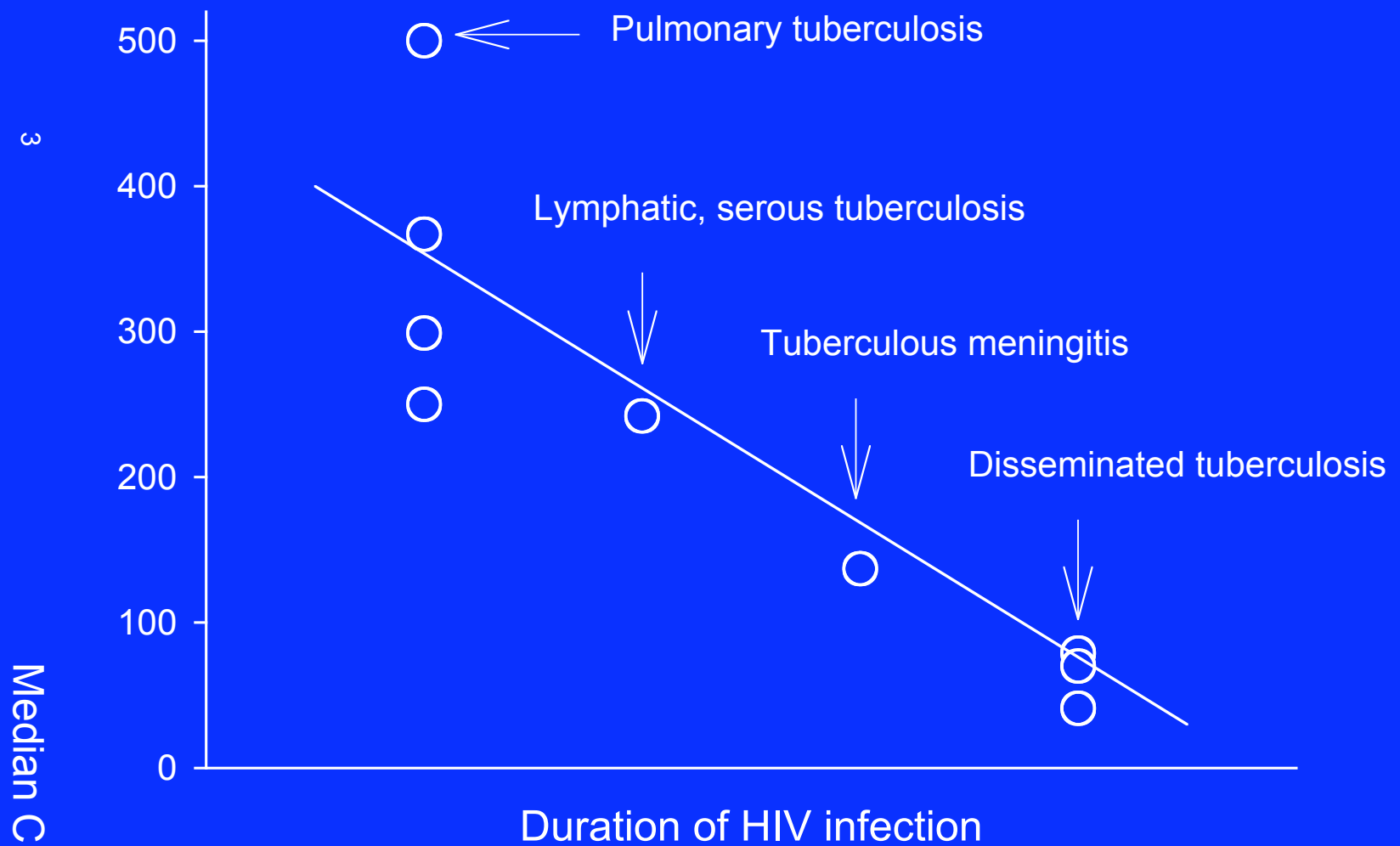




Spread of organisms in the host

- Tubercle spreads by direct extension to lymphatics → lymph nodes → blood stream → various organs
- Blood stream involvement also due to erosion of a vein
- If contents of tubercle are swallowed could be passed to stomach and intestine

Correlation Between Extent of HIV-Induced Immuno-Suppression and Clinical Manifestation of Tuberculosis



De Cock KM, et al. *J Am Med Assoc* 1992;268:1581-7

DIAGNOSIS

1. Evidence of infection

- a. Chest x-ray - hilar lymphadenopathy
calcification of primary focus/LN
- b. Delayed hypersensitivity response to purified protein
derivative (PPD) MANTOUX /HEAF TEST

2. Evidence of active disease

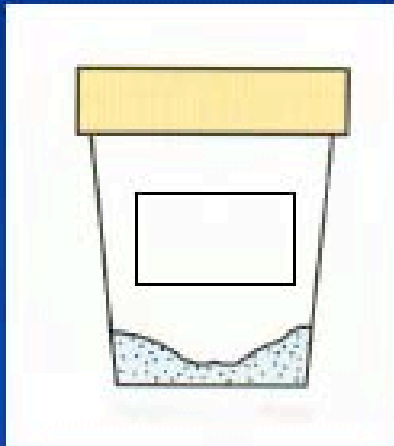
- a. Sputum for AFB positive

3. Evidence of active disease

- a. Indirect evidence of infection (Mantoux)
- b. Direct evidence of infection PCR / culture
- c. Histo-pathological evidence

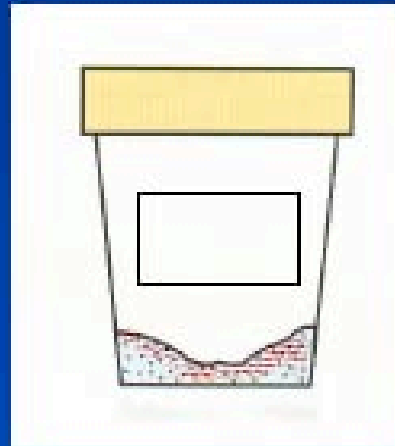
Specimen Collection (DOTS Programmes)

Day 1

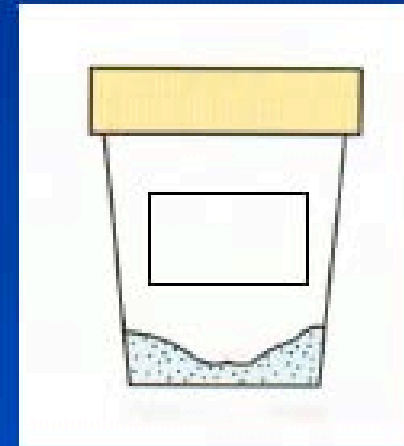


spot
(clinic)

Day 2



early a.m.
(home)

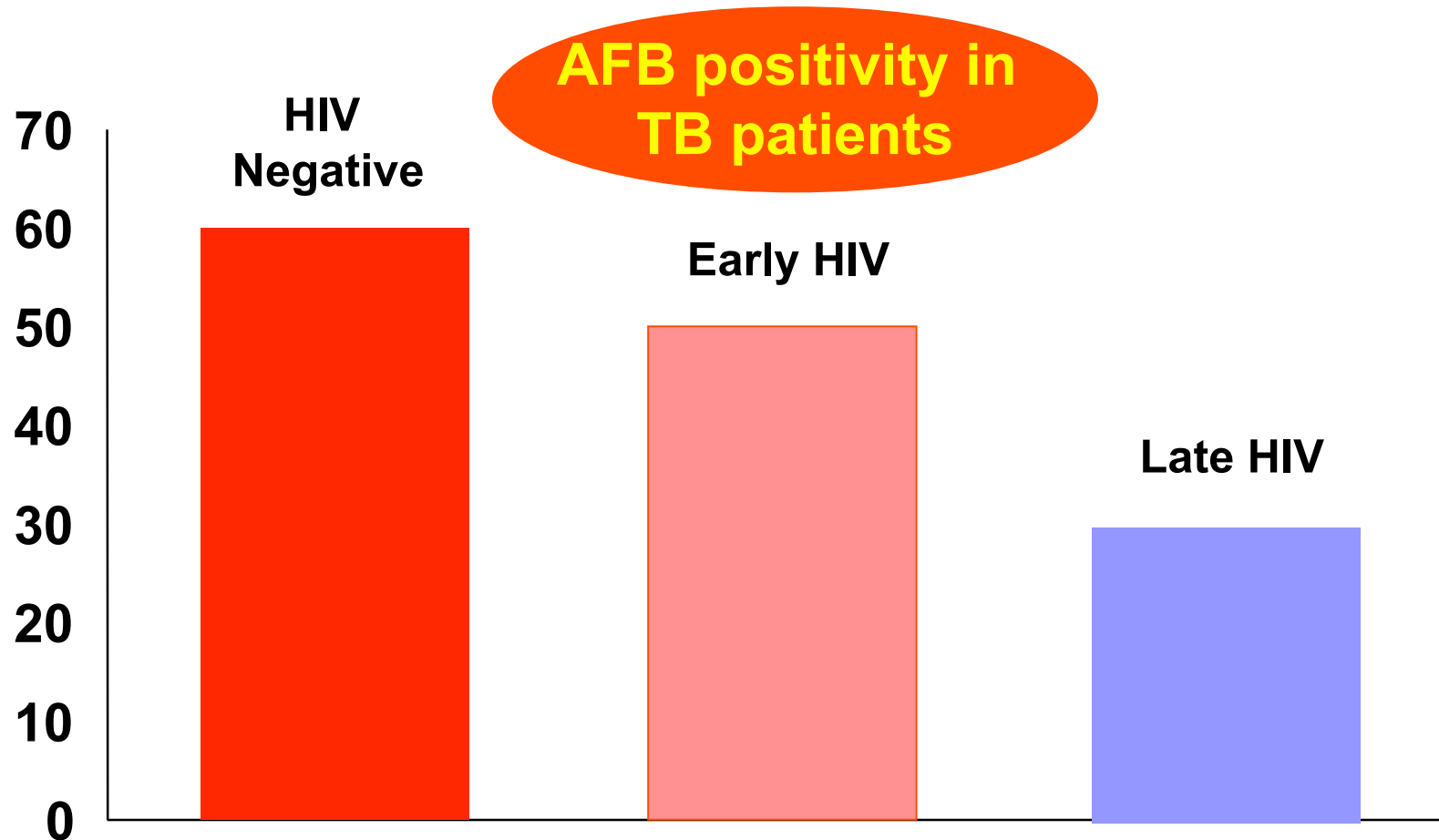


spot
(clinic)

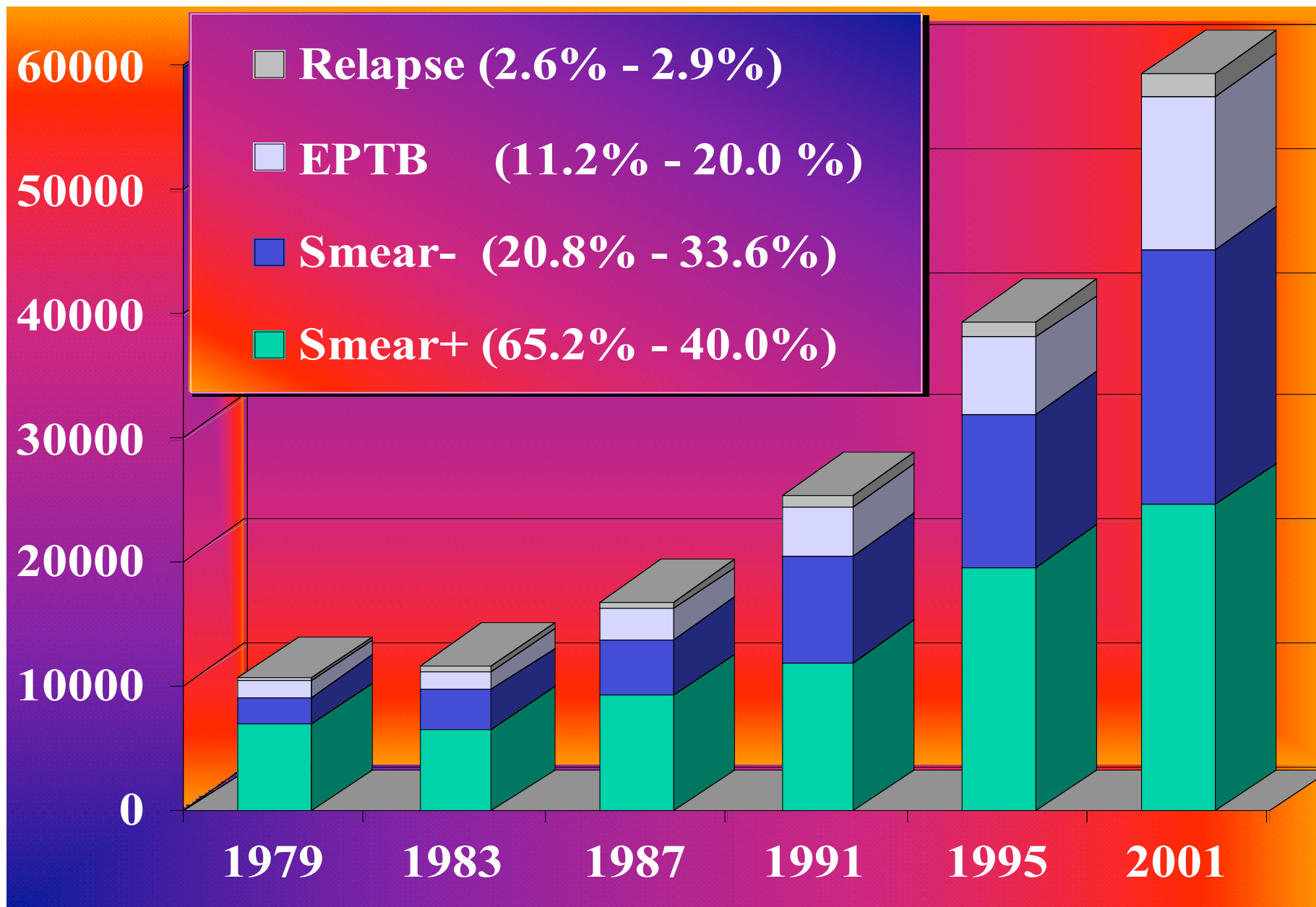
Dar Dar results

- The incremental diagnostic value was 92.1%, 1.8% and 7.1% for the first, second and third smears, respectively

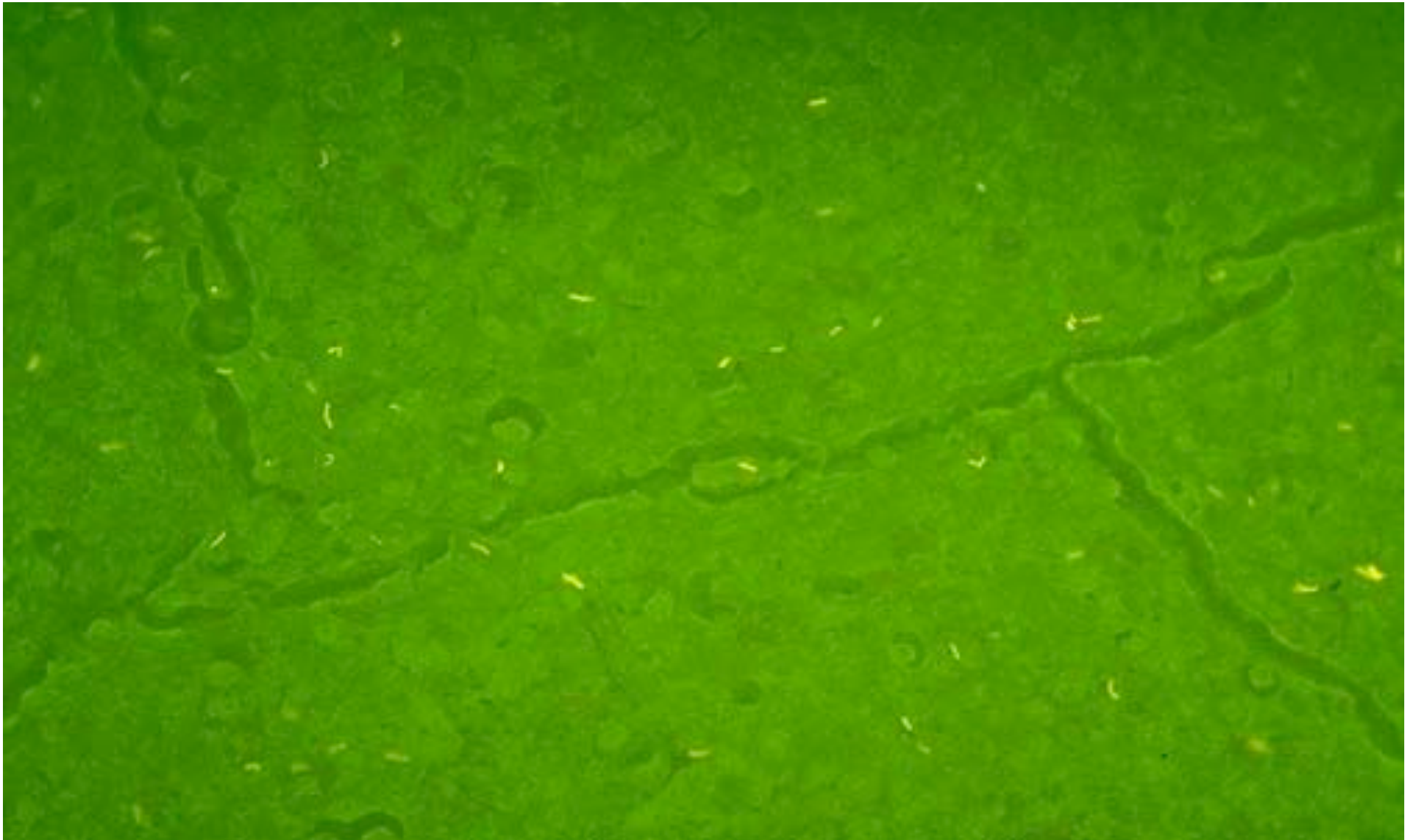
Proportion of pulmonary TB patients with positive AFB smears

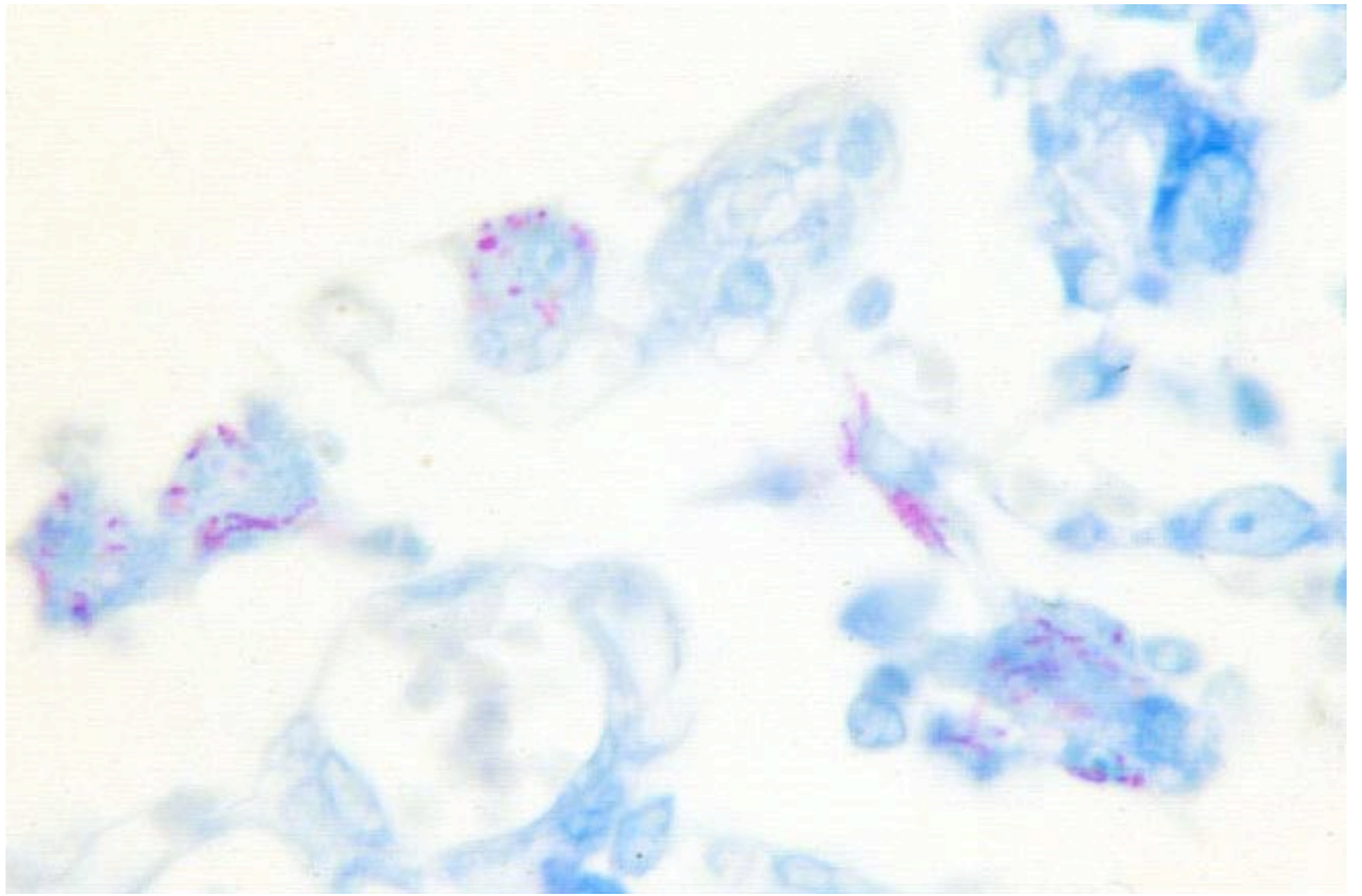


Tanzania: TB Cases - trends, 1979 to 2001



Mycobacteria: auramine stain





Sources of Error in Acid-fast Microscopy

False NEGATIVE

- poor specimen quality
- faulty smear preparation
- poor quality carbol fuchsin
- carbol fuchsin exposure too short
- technician inexperience

Sources of Error in Acid-fast Microscopy

False POSITIVE

- AFB contamination
 - ◆ container
 - ◆ slide
 - ◆ specimen carryover
 - ◆ carbol fuchsin solution
- artefact in specimen/smear
- technician inexperience



Relationship of sputum smear (SM) and sputum culture (SC) results of HIV-infected individuals

	SC positive	SC negative	Total
• SM positive	131	24	155
• SM negative	81	9108	9189
• Total	212	9132	9344

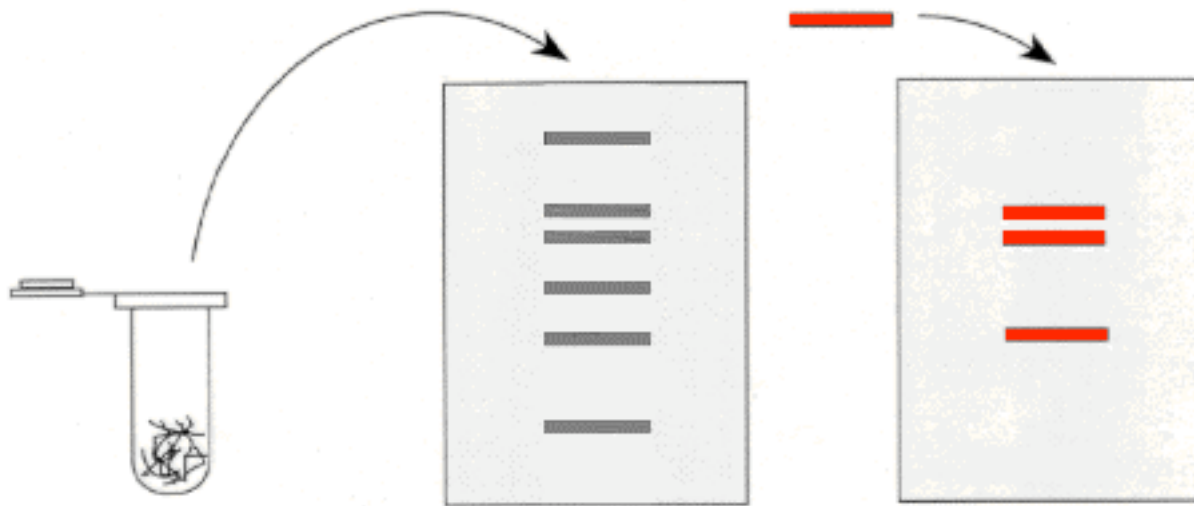
- Sensitivity = $131/212 = 61.8\%$
- Specificity = $9108/9132 = 99.7\%$
- PPV = $131/155 = 84.5\%$
- NPV = $9132/9344 = 97.7\%$

Relationship between smear and culture positivity

Culture positive	% smear positive
< 20 col/slant	22.6%
1+ (20-100 colonies/slant)	50%
2+ (discrete innumerable colonies/slant)	82.1%
3+ (confluent growth)	94.2%
Total	61.8%

Nucleic Acid Hybridization

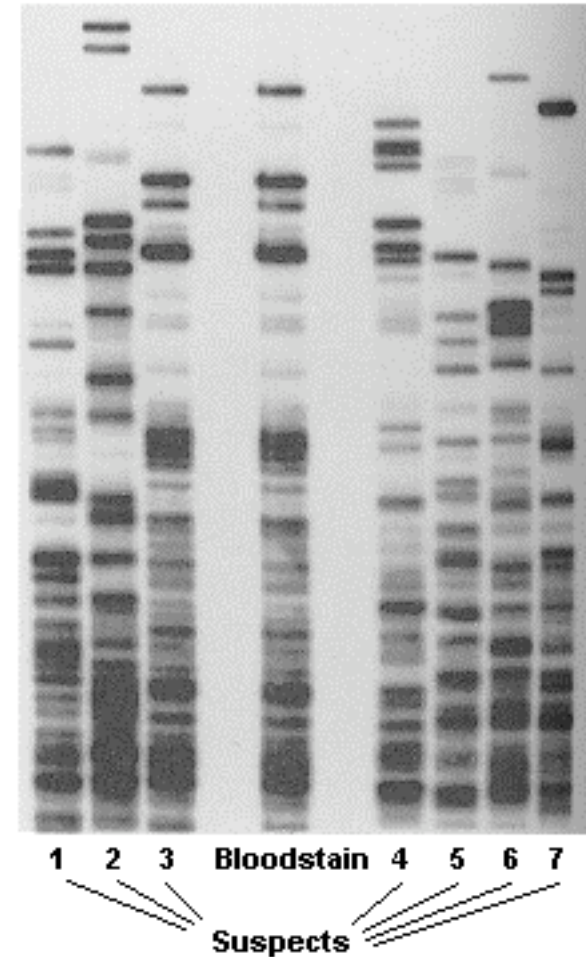
- **Ability of DNA strands from one organism to hybridize with the DNA strands of another organism**
- **Southern blotting**



DNA fingerprinting

Steps:

1. Digest DNA into smaller fragments
2. Separate fragments by size
3. Visualize bands
4. Each band corresponds to 1 fragment size
5. Unique pattern of bands identifies donor of DNA



The genetic diversity of *Mycobacterium tuberculosis* in Dar es Salaam, Tanzania, assessed by spoligotyping

- One hundred forty-seven pulmonary isolates from consecutive tuberculosis patients in Dar es Salaam were spoligotyped. SpolDB4 and 'Spotclust' were used to assign isolates to families, subfamilies and variants.
 - The Central Asian **CAS** (37%),
 - Latin American Mediterranean **LAM** (22%) and
 - East-AfricanIndian **EAI** (17%) families were the most abundant.
- This study demonstrated that the extensive TB epidemic in Dar es Salaam, Tanzania was caused by a few successful *M. tuberculosis* families, dominated by the CAS family. Import of new strains was a minor problem.
- ***BMC Microbiol.* 2006 Sep 13;6:76.**

DST results of Mtb isolated from newly diagnosed cases in Dar

- Total = 136
- Sensitive = 127 (93.4%)
- Any resistance = 9 (6.6%)
- INH = 4 (3.0%)
- SM = 3 (2.2%)
- HSE = 1 (0.7%)
- HRSE = 1 (0.7%)

- *Thank you*