



Sero-Diagnosis of TB

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Mycobacterium tuberculosis

- **Belong to family *Mycobacteriaceae***
- **Produce characteristic long chain fatty acids around 60-90 carbons in length**
- **Very slow growers**
- **Obligate aerobe, non-motile and pleomorphic rods**
- **Unusual cell wall consist of a high lipid content & acid fast. Mycolic acid makes much greater resistance.**

Introduction

- * **Infects one third of world population..!**
3 million deaths due to TB every year.
- * **Under privileged population.**
- * **Crowding, poverty and malnutrition.**
- * **Since 1985 incidence is increasing in west AIDS, diabetic, Immuno-suppressed patients and drug resistance.**



Prins Frans GUSTAF Oscar Sista stunder
1844 1851 1852 1853

The brother of King Oskar II died from tuberculosis in 1852, age 25.

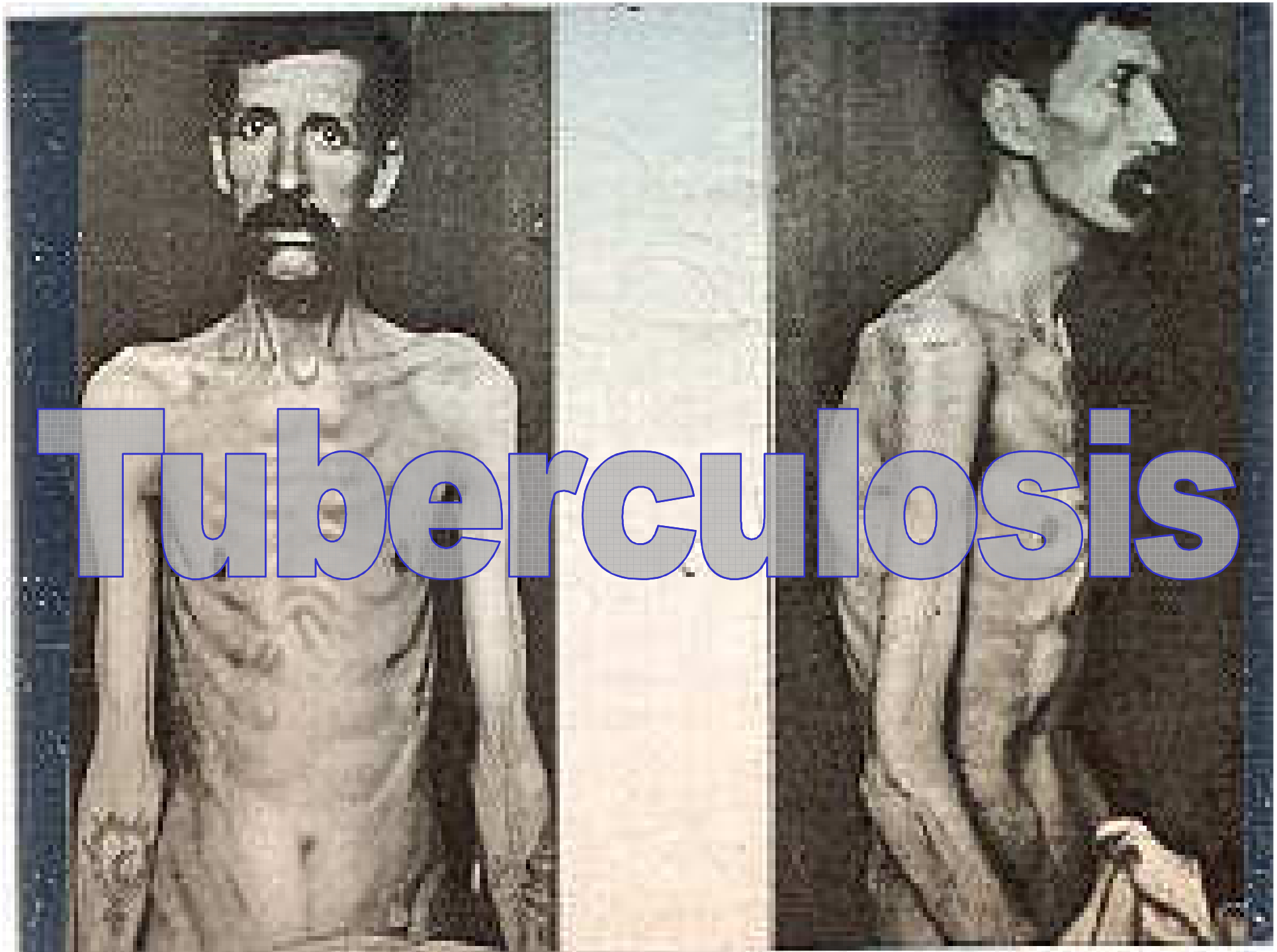
FERRYBOAT USED AS TUBERCULOSIS CAMP

837-12





TUBERCULAR SANATORIUM
HOWELL, MICH.





*Salotto della Sala di Subordinazione
S. Maria della Misericordia - 1880*



Bed rest and fresh air were important parts in the treatment of tuberculosis.

National Program for TB

- ❑ Started in 1970

- ❑ Final Aim: Get rid of TB & Control it

National Program for TB

Aim Steps:

- Increase curing rate by 2000 to more than 85%
- Early Diagnosis for more than 70% of TB cases
- Decrease infection rate to 1 / 100000 by 2010

Situation in Arab Gulf Countries

- ✓ **Ist action all over the world to get rid of TB**
- ✓ **Decrease infection rate to 1 / 100000 by 2010**
- ✓ **Use DOTS (Curing rate 90%)**

National Program Strategies for TB

- ❖ **Determine Risk groups**
- ❖ **Vaccination continuation**
- ❖ **Treatment using short plane**
- ❖ **Free Medication**

مهام مشفى الصدر وحدة الصدر ومراكز الدرن

1- استقبال الحالات والتشخيص ووضع خطة العلاج

2- تبليغ المنسق بالمديرية بالحالات المكتشفة

3- اخطار المراكز الصحية لعمل التقصي الوبائي

4- استمرار متابعة المرضى غير المنومين

5- عمل اختبار نقص المناعة المكتسب لجميع

المرضى

6- ارسال الاحصائيات الدورية والتقارير الى المديرية

مهام مراكز الرعاية الصحية الأولية

- ١- التعرف على الحالات المشتبهه وتحويلها
- ٢- ضمان وصول الحالة الى الجهة المحولة
- ٣- عمل الاستقصاء الوبائي حصر وتوجيه المخالطين
- ٤- متابعة اعطاء العلاج
- ٥- تدريب أحد أقارب المريض لملاحظة المريض

www.globalfundatm.org

The Global Fund to Fight AIDS, Tuberculosis and Malaria - Microsoft Internet Explorer provided by WHO

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THE ISSUES

The Global Fund to Fight AIDS, Tuberculosis & Malaria

Funding the fight against the diseases of poverty



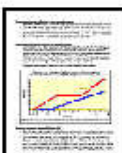
Annual Report 2002/2003

Download the [Executive Summary](#) (PDF 69 Kb) of the Global Fund's 2002/2003 Annual Report or [write to us](#) to receive a full hard copy.



September 2003 Progress Report

[English](#) 153 Kb



(PDF)

Download our new brochure

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LATEST NEWS

[Impact of Global Fund Begins to Be Felt in Asia and the Pacific](#) (29 September 2002)
A "Treat Asia Report" interview of Dr. Richard Feachem.

[The Global Fund Substantially Increases Funding for AIDS](#) (22 September)

[Global Fund Disbursements Top US\\$100 Million](#) (29 August)

[South Africa Grant Agreements Signed: Over US\\$ 40 Million for Treatment and Prevention of HIV/AIDS and TB](#) (7 August 2003)



[UNAIDS and Global Fund sign memorandum of understanding](#) (4 August 2003)

[Presidents of the European Commission and France commit to a billion dollars from](#)

[The Global Fund in Action and other Highlights](#)

Internet

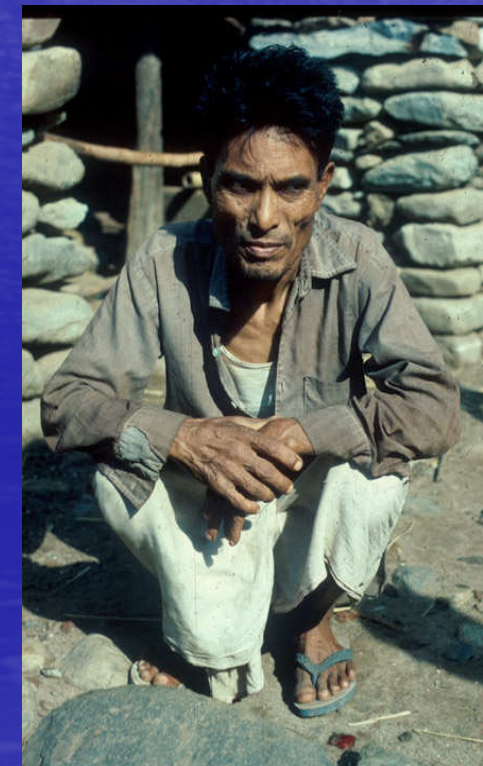
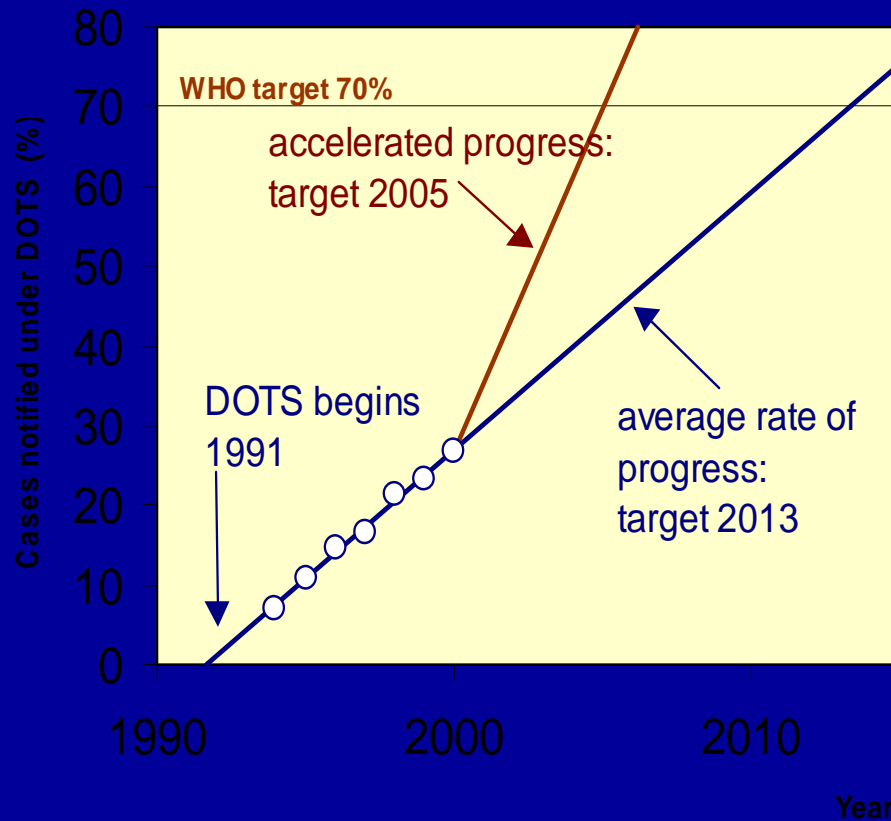
Situation in KSA

➤ Infection rate:

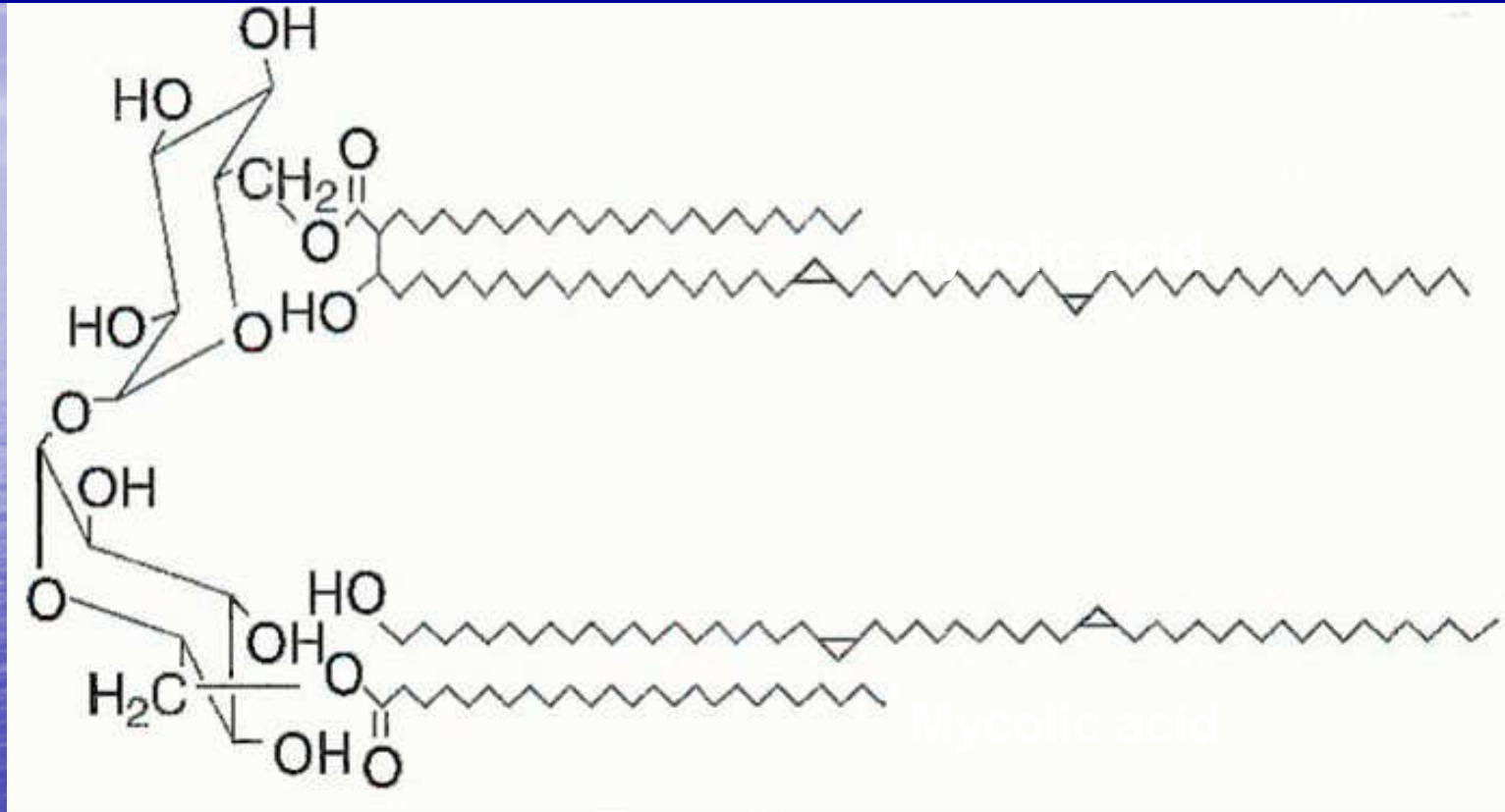
➤ 1987 rate 30.5 /100000

➤ 1996 rate 11.6 /100000

The Need for a Global Drug Facility



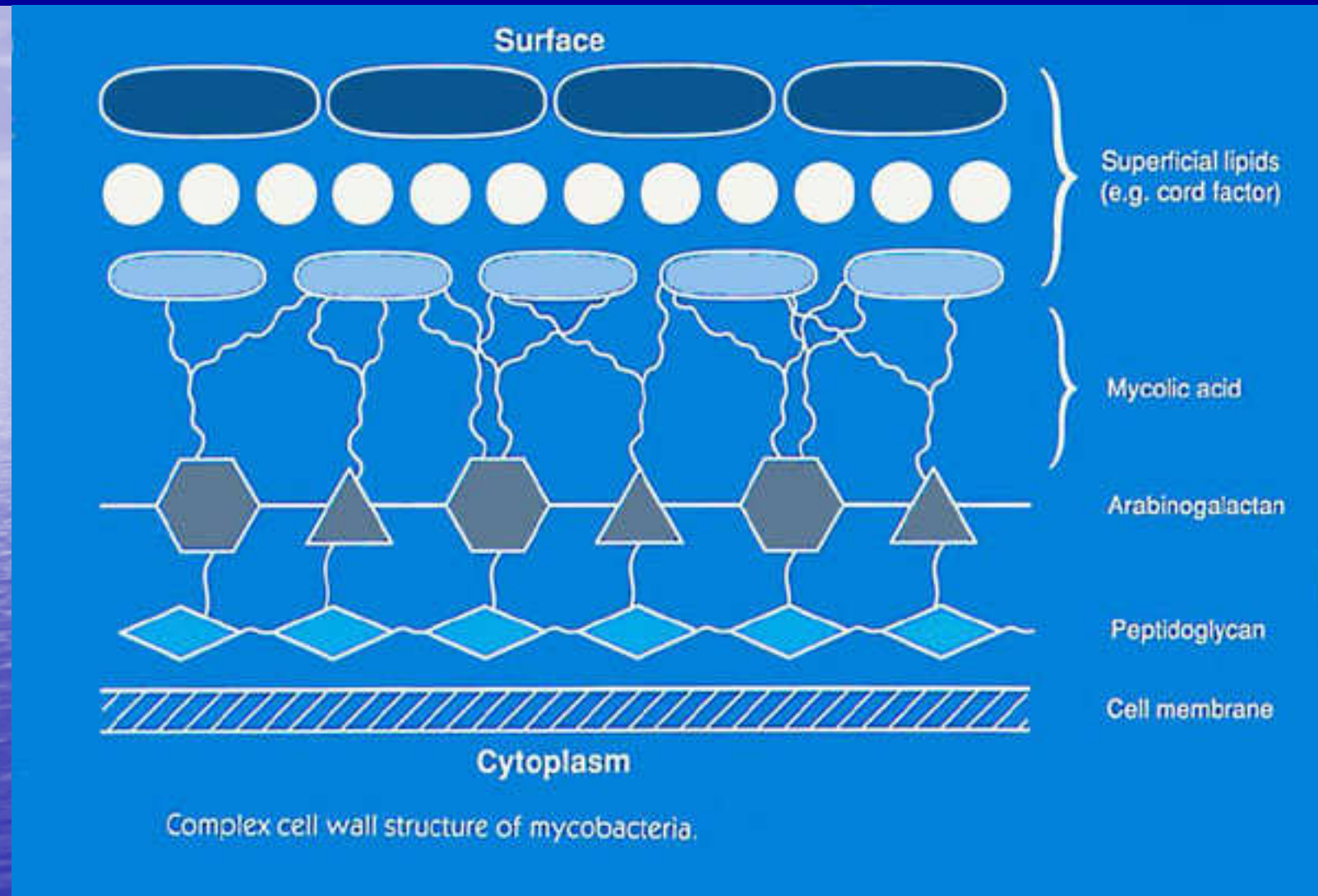
6,6'-TREHALOSE DIMYCOLATE (TDM) “CORD FACTOR”



CELL WALL GLYCOLIPID FROM *MYCOBACTERIUM*

A possible VIRULENCE FACTOR for *M. tuberculosis*

CELL ASSOCIATED TOXINS MAY BE IMPORTANT VIRULENCE FACTORS FOR MANY MYCOBACTERIA



The glycolipids, such as trehalose dimycolates, of *Mycobacterium tuberculosis* and related organisms appear to be related to their virulence

TB INFECTION SPRED

- * **Communicable disease caused by tubercle bacillus.**
- * **Spread by air droplet nuclei.**
- * **Infection begins with multiplication of tubercle bacilli.**
- * **Some of bacilli spread through the bloodstream.**
- * **Immune system contains bacilli-prevents disease and persons are infected but do not have disease and remain symptomatic and not infectious.**

COVER UP!

YOUR COUGHS AND SNEEZES

Actual photograph of a sneeze



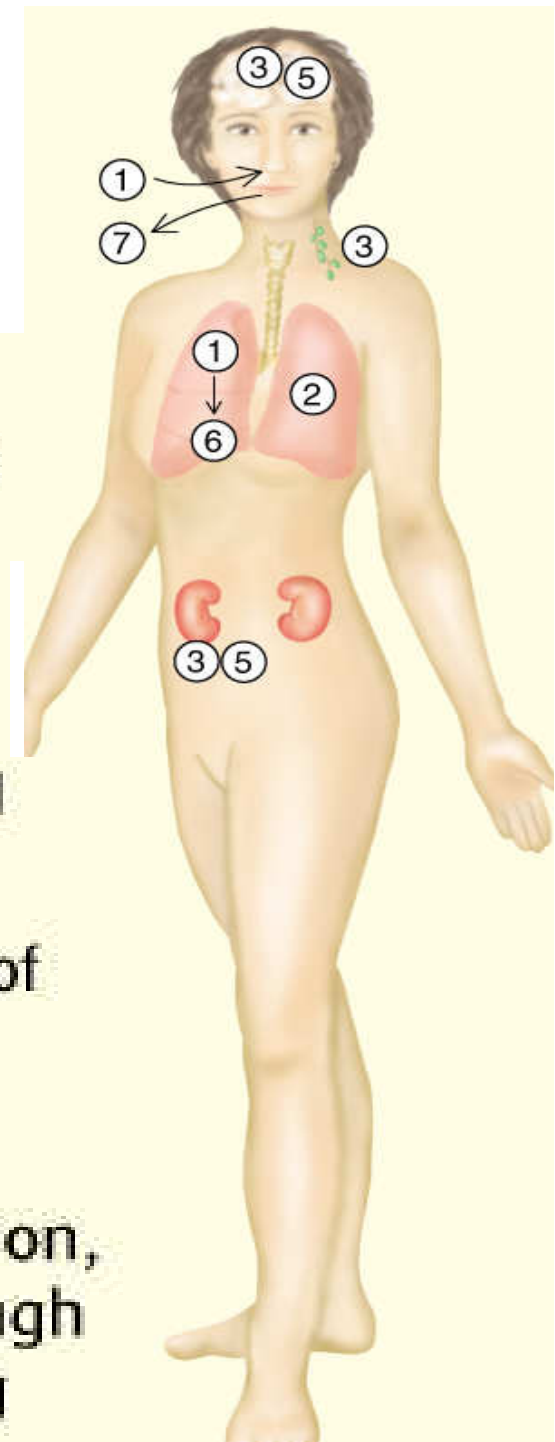
SPRAY SPREADS
COLDS • FLU • TUBERCULOSIS

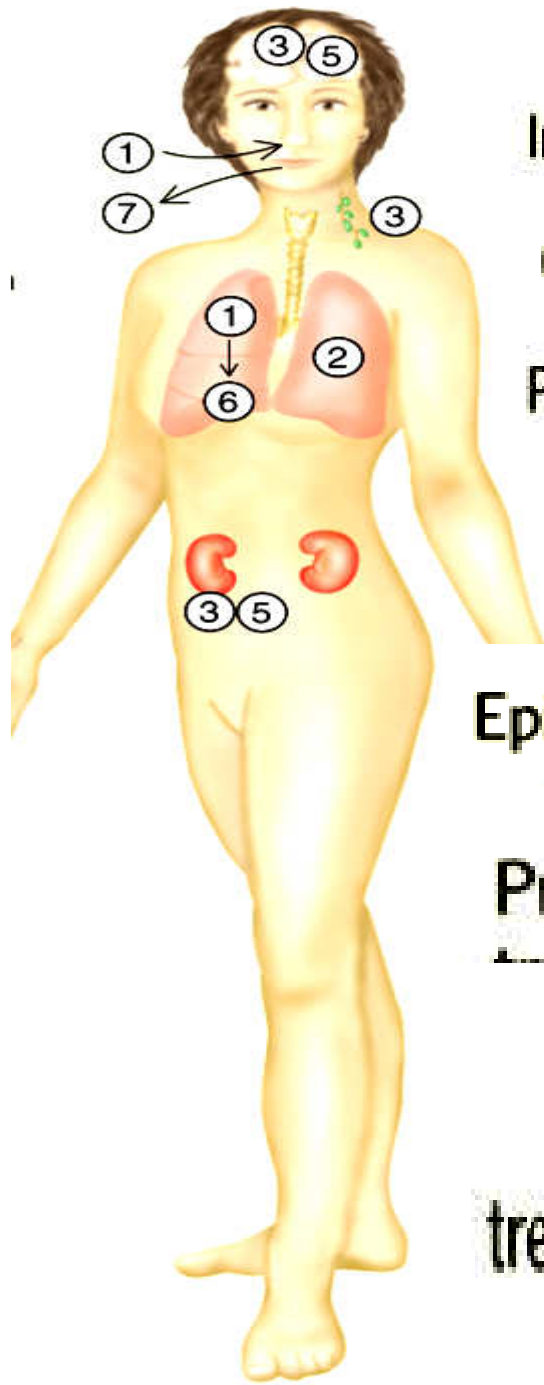
TB BODY LOCATIONS

Can infect (disseminate) and cause disease in many different body locations such as:

- 1. Meninges**
- 2. Brain**
- 3. Bone**
- 4. Kidney**
- 5. Essentially any organ (lung primary target)**
- 6. Other locations: e.g. ...**

- ① Airborne *Mycobacterium tuberculosis* bacteria are inhaled and lodge in the lungs
- ② The bacteria are phagocytized by lung macrophages and multiply within them, protected by lipid-containing cell walls
- ③ Infected macrophages are carried to various parts of the body such as the kidneys, brain, lungs, and lymph nodes; release of *M. tuberculosis* occurs
- ④ Delayed hypersensitivity develops; wherever infected *M. tuberculosis* has lodged, an intense inflammatory reaction develops
- ⑤ The bacteria are surrounded by macrophages and lymphocytes; growth of the bacteria ceases
- ⑥ Intense inflammatory reaction and release of enzymes can cause caseation necrosis and cavity formation
- ⑦ With uncontrolled or reactive infection, *M. tuberculosis* exits the body through the mouth with coughing or sneezing





Symptoms

Fever, weight loss, cough, sputum production

Incubation period

2 to 10 weeks

Causative agent

Mycobacterium tuberculosis

Pathogenesis

Colonization of the alveoli incites inflammatory response; ingestion by macrophages follows; organisms survive ingestion and are carried to lymph nodes, lungs, and other body tissues; tubercle bacilli multiply; granulomas form

Epidemiology

Inhalation of airborne organisms; latent infections can reactivate

Prevention & treatment

BCG vaccination, not used in the United States; tuberculin (Mantoux) test for detection of infection, allows early therapy of cases; treatment of young people with positive tests and individuals whose skin test converts from negative to positive. Treatment:

treatment

two or more antitubercular medications given simultaneously, such as isoniazid (INH) and rifampin

Clinical Presentation: Pulmonary

- * **Cough** - one of the earliest and most common symptoms present in 40-80%
- * **Sputum production-cough**
nonproductive initially, but later productive (reflecting tissue necrosis)
- * **Pleuritic chest pain** - inflammation of pleura

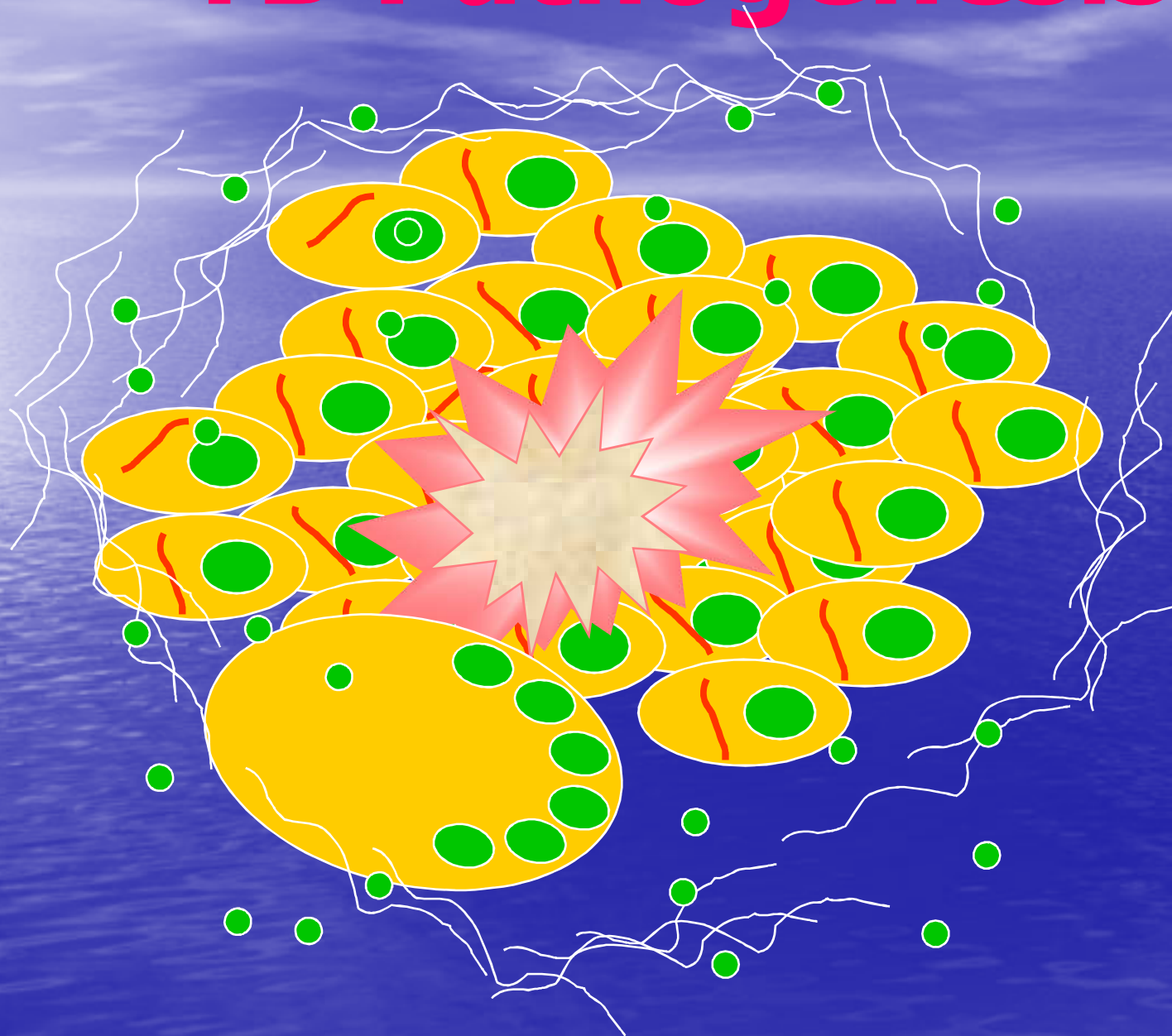
Clinical Presentation: Pulmonary (Cont')

- * **Dyspnea**- late symptom, may indicate extensive disease.
- * **Hemoptysis**- may or may not indicate active disease. More common with bronchitis.

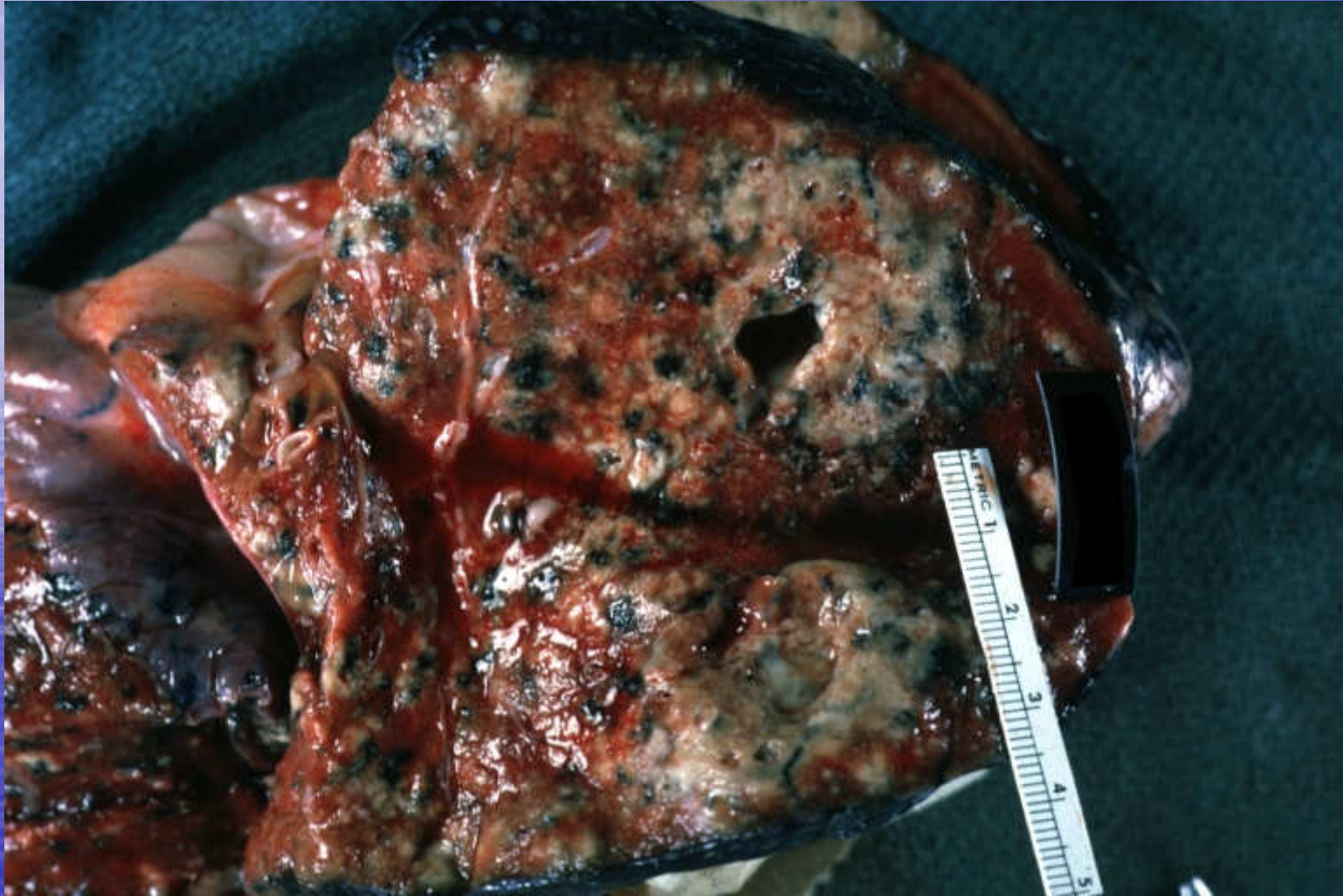
Pathogenesis of TB

- * **Type IV hypersensitivity – T cells – Macrophages → Granuloma**
- * **Activated macrophages – epithelioid cells. Remain viable inside Macrophages**
- * **Mycolic acid wax coat. Cord Factor - surface glycolipid antigenic. Self destruction by lysosomal enzymes.**

TB Pathogenesis



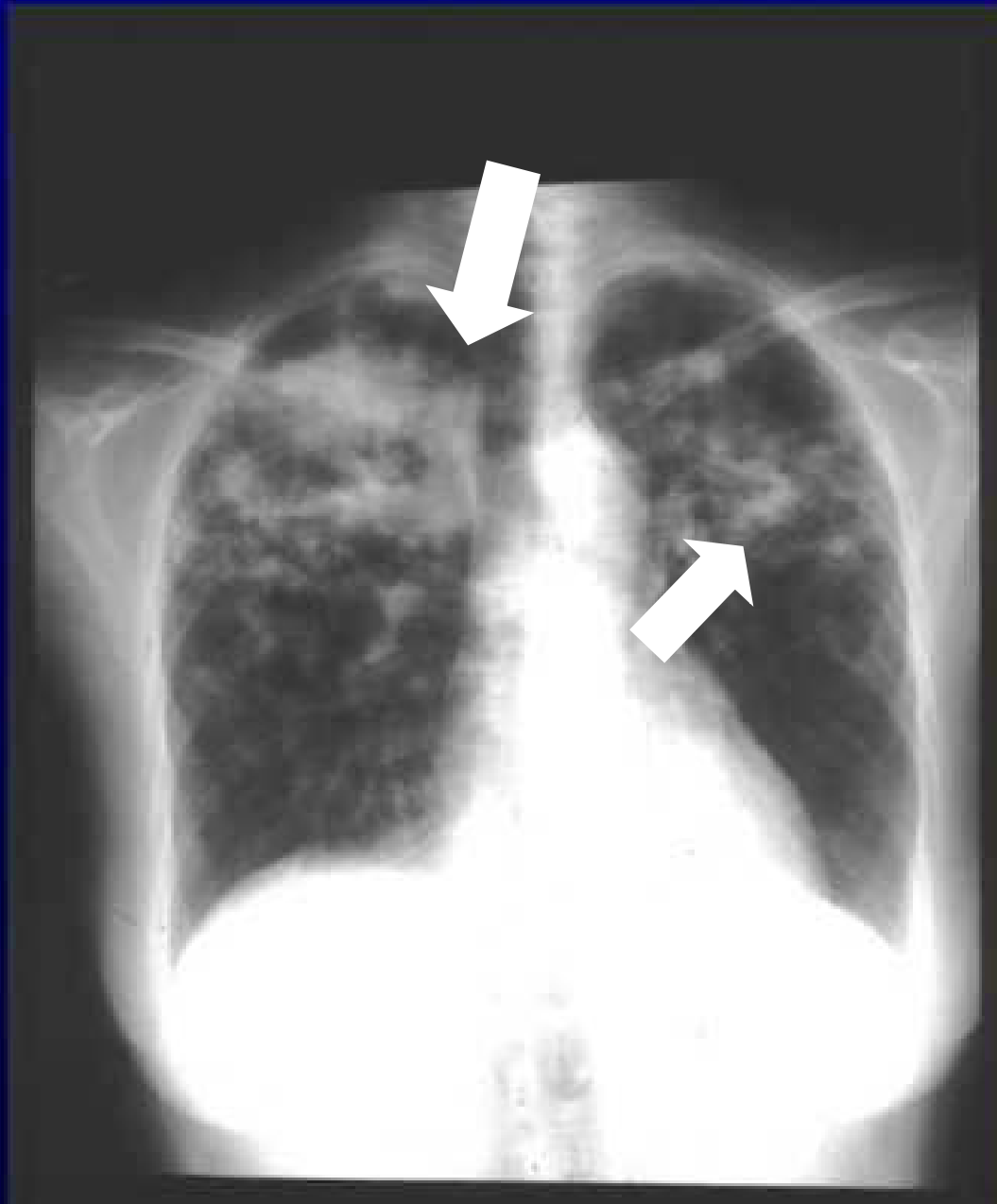
Lung TB - Cavitation



TB - Scrofula (Mexico)



Untreated Tuberculosis CXR



- Rate of having false-positive cultures resulting from laboratory cross-contamination may be up to 33% of culture-confirmed TB patients.
- The average cost per patient is \$10,873 (range, \$1,033-\$21,306).
- © 2006(CDC)

Sputum collection



Collection of sputum sample

- Select a wide-mouthed plastic container, disposable, unbreakable and leak proof material.
- Give the patient a sputum container with the laboratory serial No. written on it. Show the patient how to open and close the container and explain the importance of not rubbing off the number written on the side of the container.
- Instruct the patient to inhale deeply 2-3 times, cough up deeply from the chest and spit in the sputum container by bringing it closer to mouth.

Collection of sputum sample (Cont')

- **Make sure the sputum sample is of good quality. A good sputum sample is thick, purulent and sufficient in amount (2-3 ml).**
- **Give the patient another container with laboratory serial number written on it for an early morning specimen.**
- **Explain to the patient to rinse his/her mouth with plain water before bringing up the sputum.**

Microscopy of sputum

- **Diagnosis of pulmonary TB by sputum microscopy is simple, easy, inexpensive, rapid, technically not very demanding and more reliable than X-rays.**
- **The purpose of the sputum microscopy is two fold (a) diagnosis of the patients with infectious tuberculosis (b) monitoring the progress.**
- **For diagnosis, 3 sputum examinations are performed (spot, morning, spot) and for follow up 2 sputum examinations (morning, spot) are performed.**

Microscopy-problems

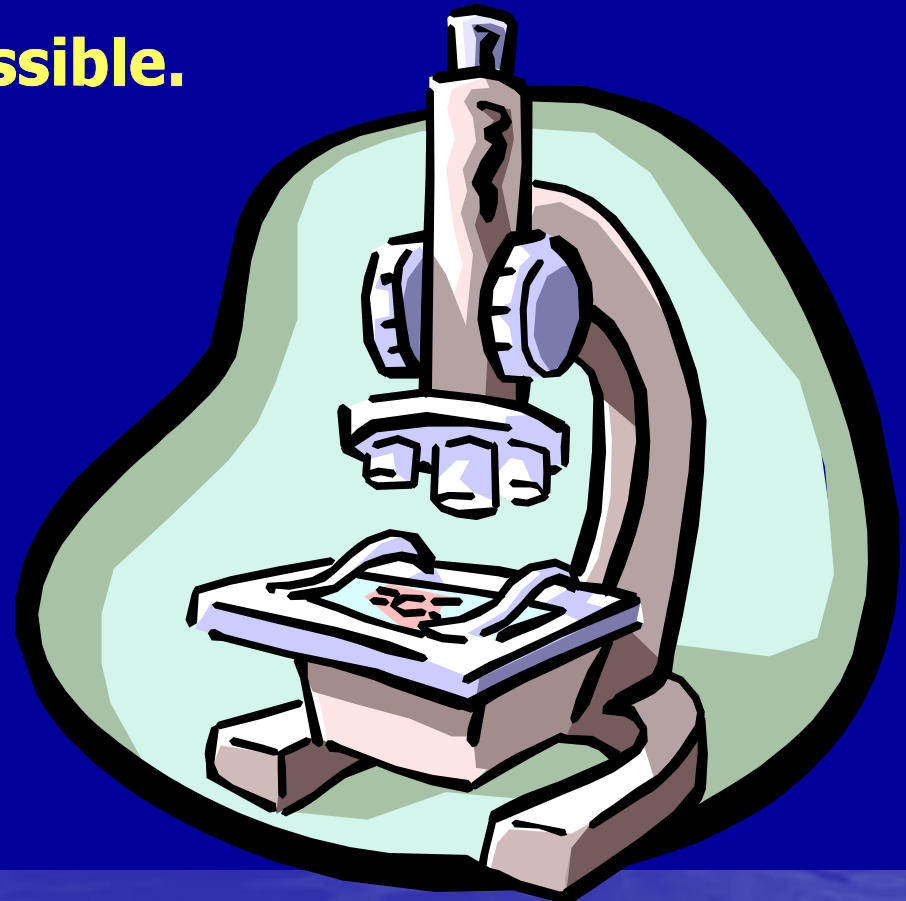
least count 5000-10000 bacilli per ml.

Species differentiation impossible.

Specimen contamination.

False positive

Saprophytic mycobacteria.



Preparation of smear and Ziehl Neelsen staining (AFB staining)

- **Unscratched new slide, label the slide with laboratory serial number.**
- **Make a smear from yellow purulent portion of the sputum using a bamboo stick.**
- **A good smear is spread evenly, 2 cm x 3 cm in size and is neither too thick nor too thin.**
- **The optimum thickness of the smear can be assessed by placing the smear on a printed matter and the print should be readable through the smear.**
- **Let the smear air dry for 15-30 minutes. Fix the smear by passing the slide over the flame 3-5 times for 3-4 seconds each time.**
- **Stain the smear by Ziehl Neelsen method.**

- **STEP 1: Flame slides to heat fix**



- **STEP 2: Flood the entire slide with Carbol Fuchsin**
- **Ensure enough stain is added to keep the slides covered throughout the entire staining step.**



STEP 3: Using a Bunsen burner, heat the slides slowly until they are steaming. Maintain steaming for 5 minutes by using low or intermittent heat (i.e. by occasionally passing the flame from the Bunsen burner over the slides).

Caution: Using too much flame or heat can cause the slide to break.



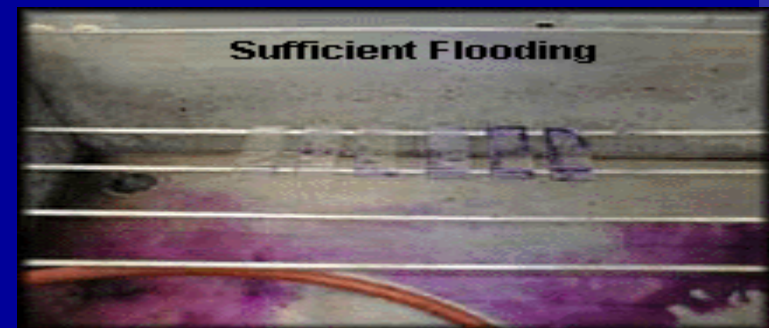
STEP 4: Rinse the slide with water.



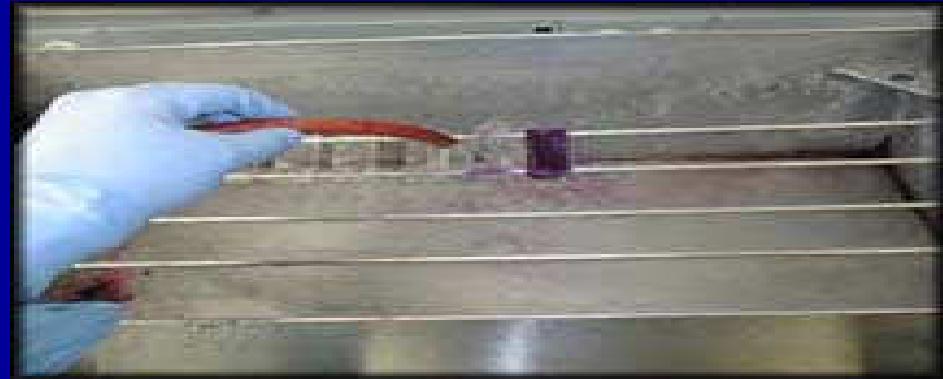
- **STEP 5: Flood the slide with 3% acid-alcohol and allow to decolorize for 5 minutes.**



- **Throughout the 5 minutes, continue to flood the slides with 3% acid-alcohol until the slides are clear of stain visible to the naked eye.**
- **To the right are examples of slides insufficiently and sufficiently flooded with 3% acid-alcohol.**



- **STEP 6:** Rinse the slide thoroughly with water and then drain any excess from the slides.



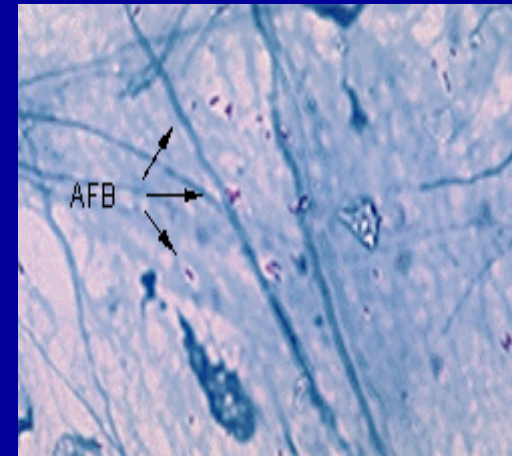
- **STEP 7:** Flood the slide with the counterstain, Methylene Blue. Keep it on the slides for 1 min.



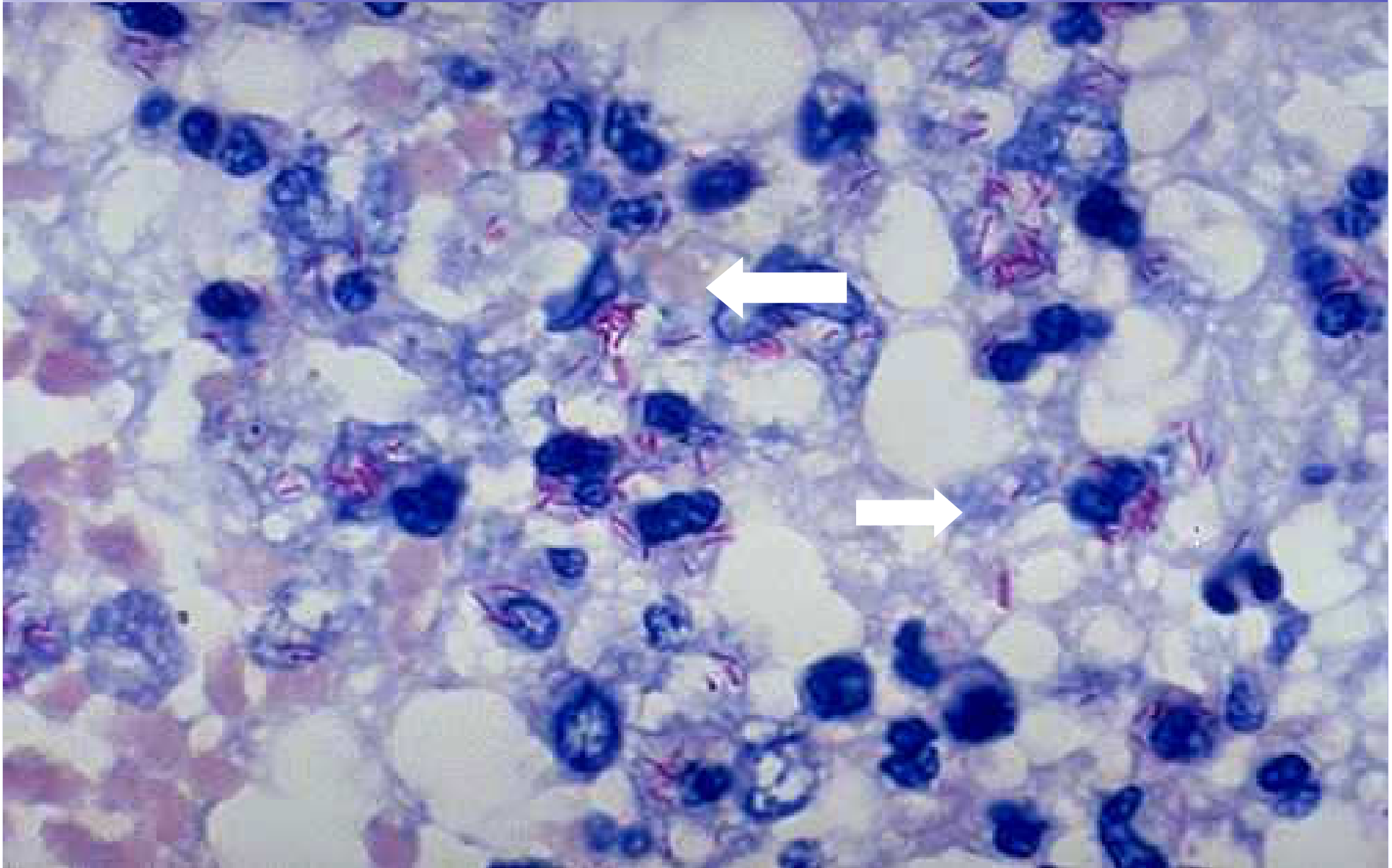
- **STEP 8: Rinse the slide thoroughly with water**



If all steps are performed correctly you should have a slide that looks like this.



AFB - Ziehl-Neelson stain



How to prevent false positive sputum results?

- **Only use new, unscratched slides. Always use filtered carbol fuchsin.**
- **Do not allow the carbol fuchsin to dry during staining.**
- **Do not allow the carbol fuchsin to boil during staining.**
- **Decolorize adequately with sulphuric acid.**
- **Make sure there are no food particles or fibres in the sputum samples.**
- **Never allow the oil immersion lens to touch a slide.**
- **Label sputum containers, slides, and laboratory forms accurately.**
- **Record and report results accurately.**

How to prevent false negative sputum results

- **Make sure the sample contains sputum and not just saliva.**
- **Make sure there is enough sputum (at least 2 ml).**
- **Select thick, purulent portions to make the smear.**
- **Prepare smears correctly – not too thick, too thin or with too little material.**
- **Fix for the correct length of time, not too short or too long.**
- **Stain with carbol fuchsin for 5-7 minutes.**
- **Do not decolorize with sulphuric acid too intensively.**
- **Examine every smear for at least five minutes observing at least 100 fields before recording as negative.**
- **Label the sputum containers, slides and laboratory forms carefully.**
- **Record and report results accurately.**

Culture for *M. tuberculosis*

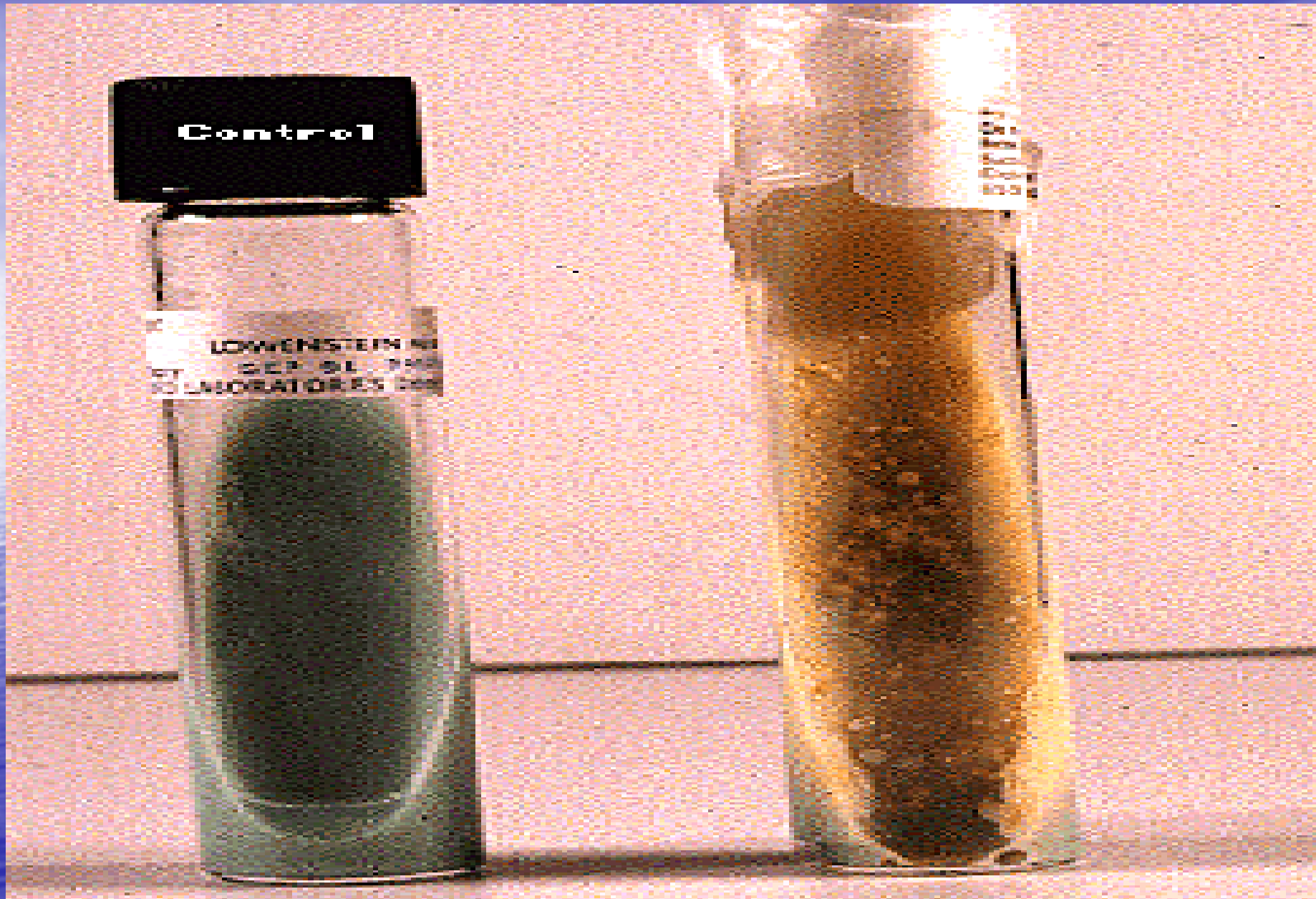
Each case of suspected tuberculosis need not be cultured. The judicious use of culture limits its use to the followings:

- 1-Diagnosis of smear negative pulmonary TB cases with strong clinical and radiological suspicion.
- 2-Diagnosis of extrapulmonary TB.
- 3- Follow up of a case to investigate for a drug resistant isolate.
- 4-Diagnosis of childhood tuberculosis.

Control

LOWENSTEIN
SEP 01 1985
COLLABORATOR'S DES

Mycobacterium tuberculosis
Lowenstein-Jensen media



Colony Morphology – L J Slant



COLONIAL MORPHOLOGY OF THE TUBERCULOSIS COMPLEX MYCOBACTERIA



EUGONIC GROWTH 14 DAYS

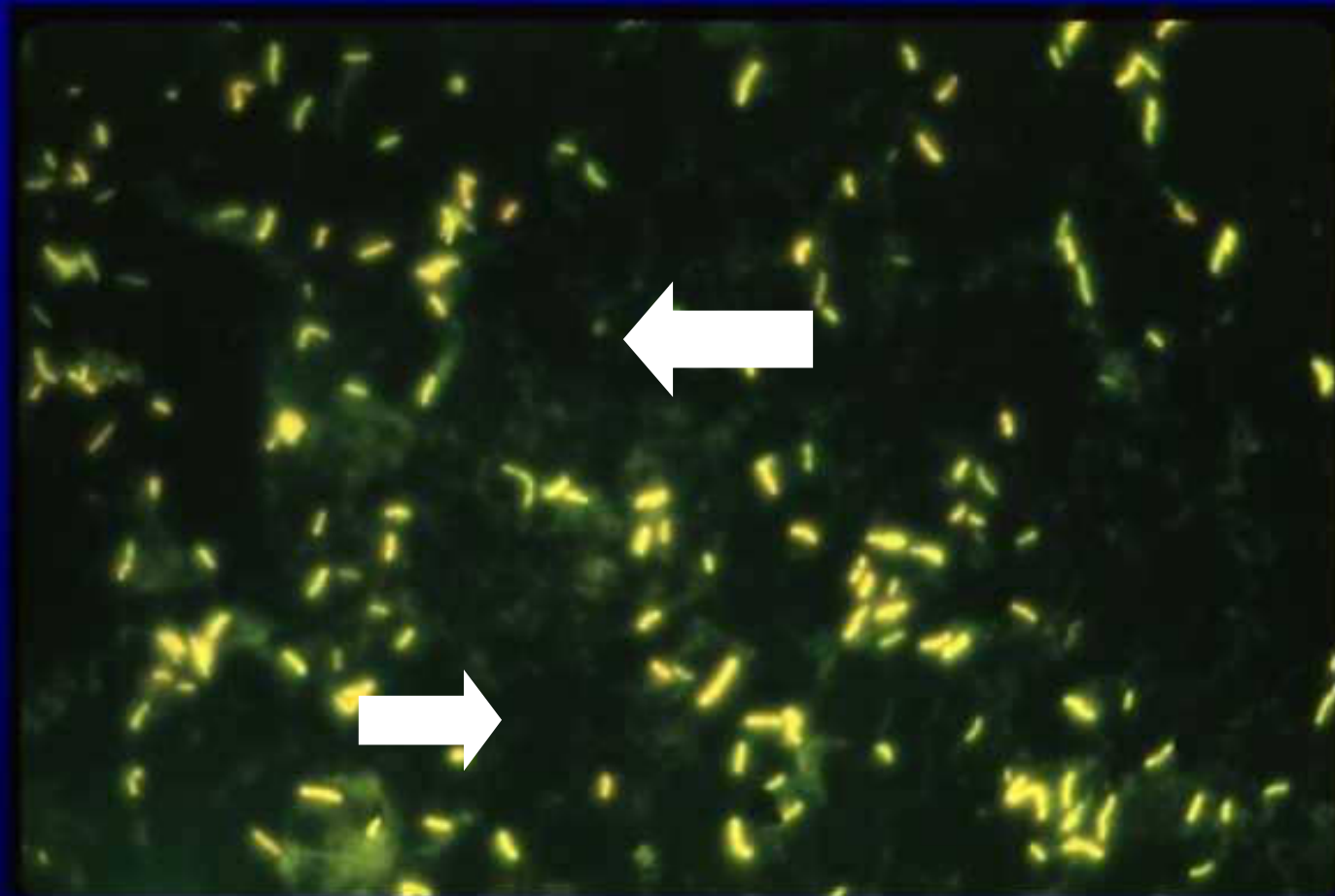
Mycobacterium tuberculosis



DYSGONIC GROWTH 14 DAYS

Mycobacterium bovis

Sputum - TB Auromine/Rhodamine



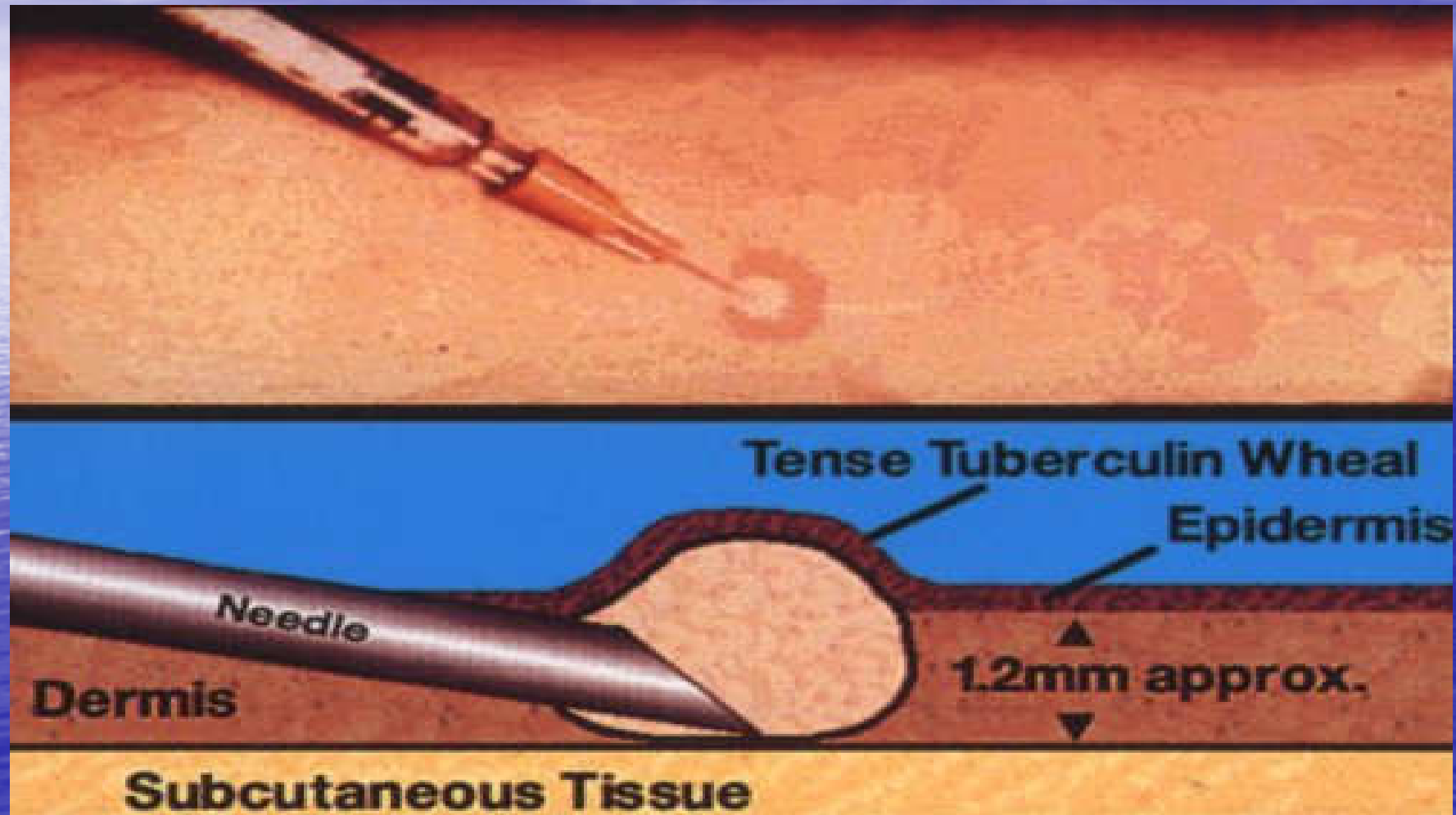
Indicators of potential false-positive M. tuberculosis cultures:

- **All** specimens from a patient are AFB smear-negative, and only one is M. tuberculosis culture-positive.
- The patient's signs, symptoms, and clinical course are **inconsistent** with TB.
- An M. tuberculosis culture-positive specimen, also likely to be AFB smear-positive, was processed the **same day** as the suspected specimen.

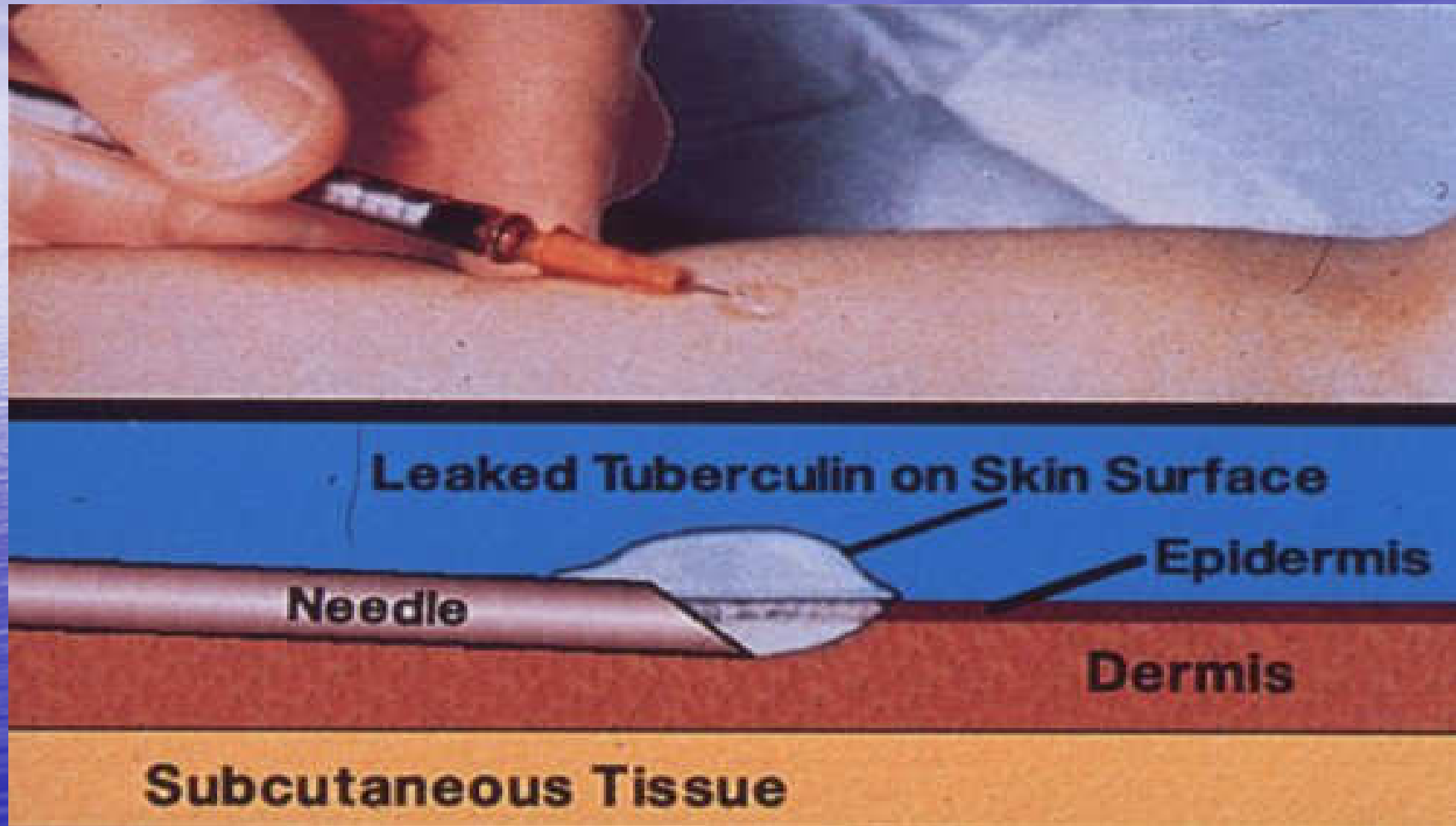
Administering the TST

- * Inject intraderamly 0.1ml of 5 TU PPD**
- * Produce wheal 6mm to 10mm in diameter**
- * Follow universal precautions**

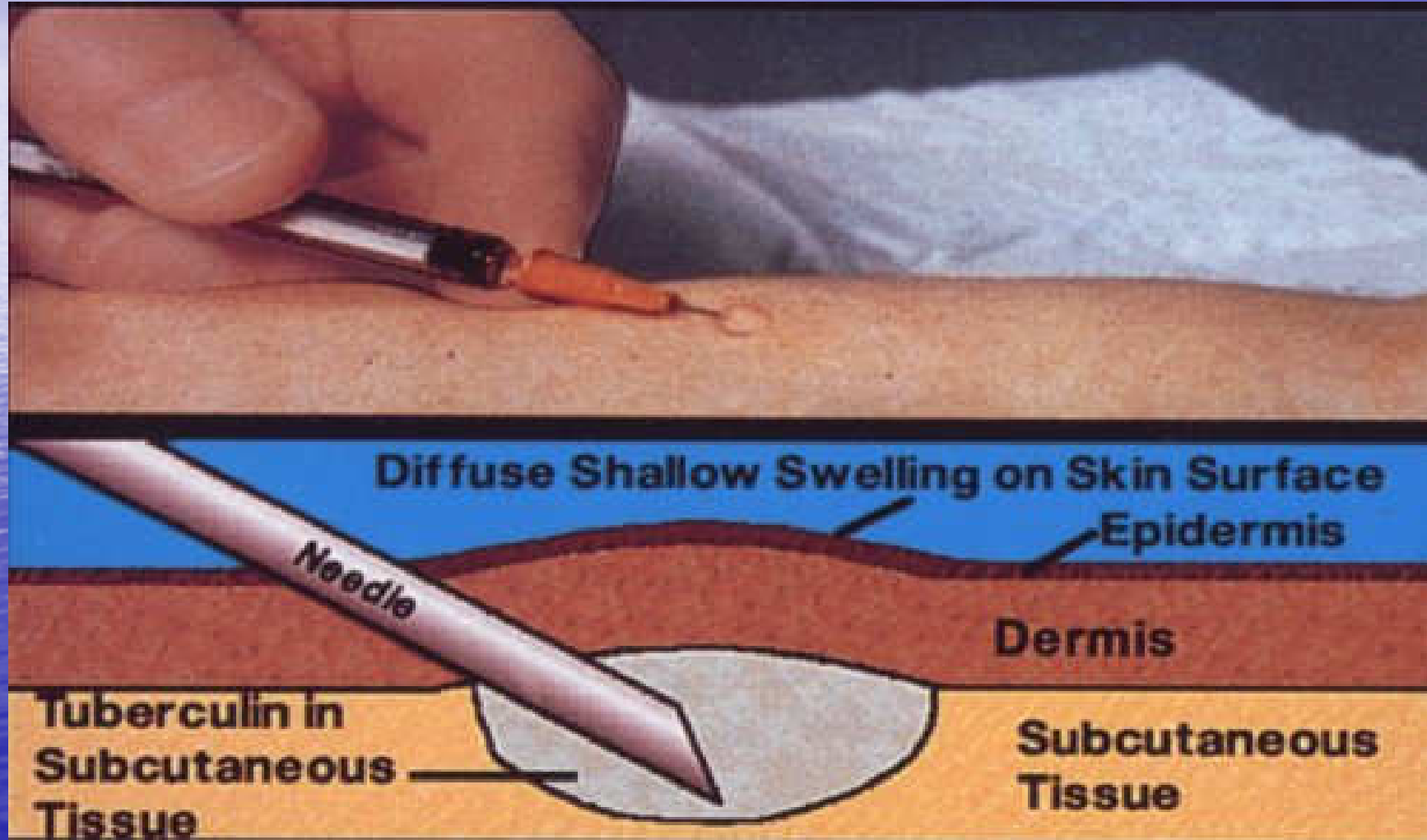
Inserted Properly



Needle too Shallow



Needle too Deep



Reading the TST

*** Read reaction 48-72 hrs after injection.**

*** Measure only induration, not erythema.**

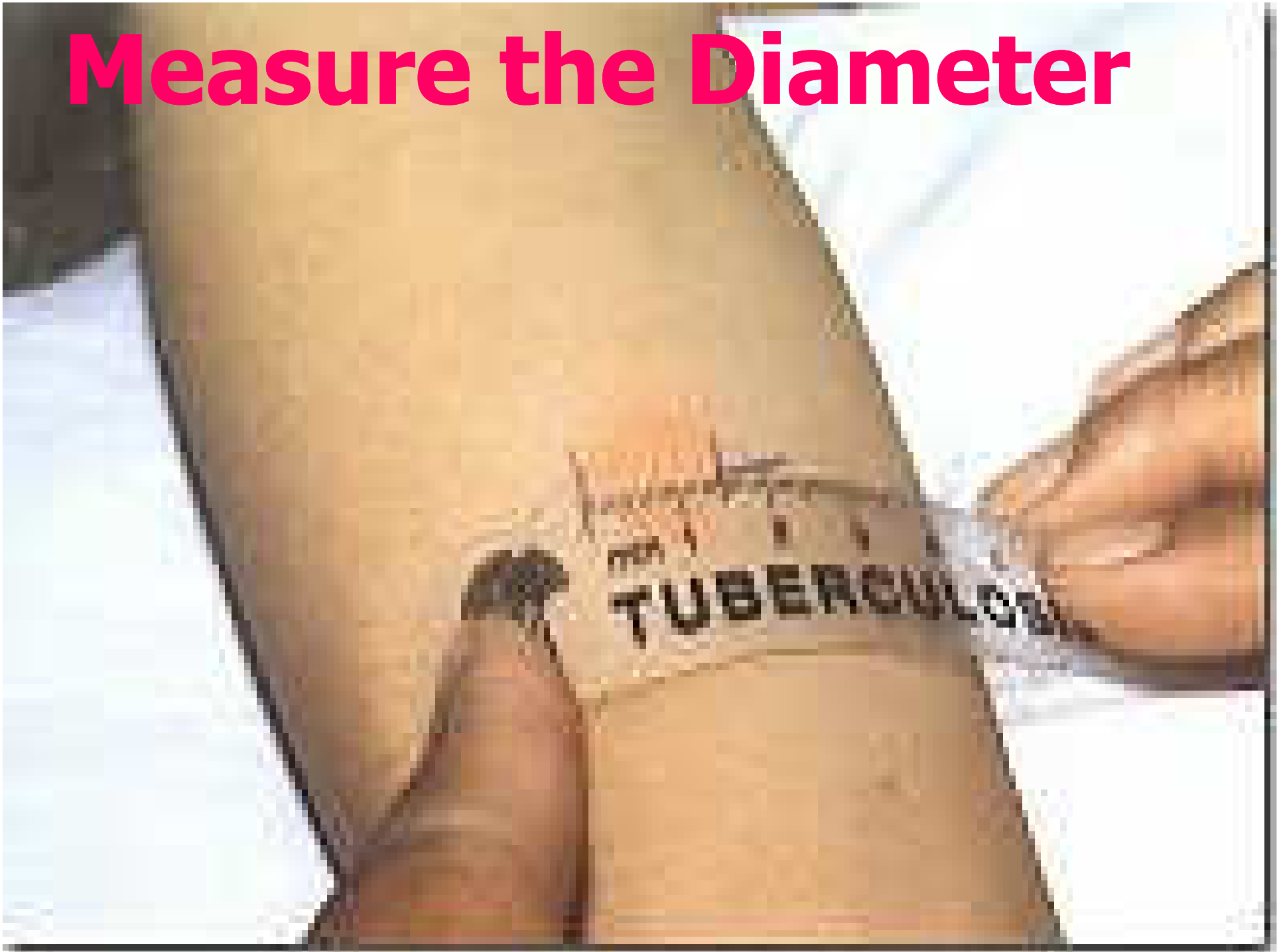
*** Record reaction in millimeters.**

**5mm- HIV infected, close contacts
immunosuppressed**

10mm- targeted testing

15mm- low risk persons

Measure the Diameter

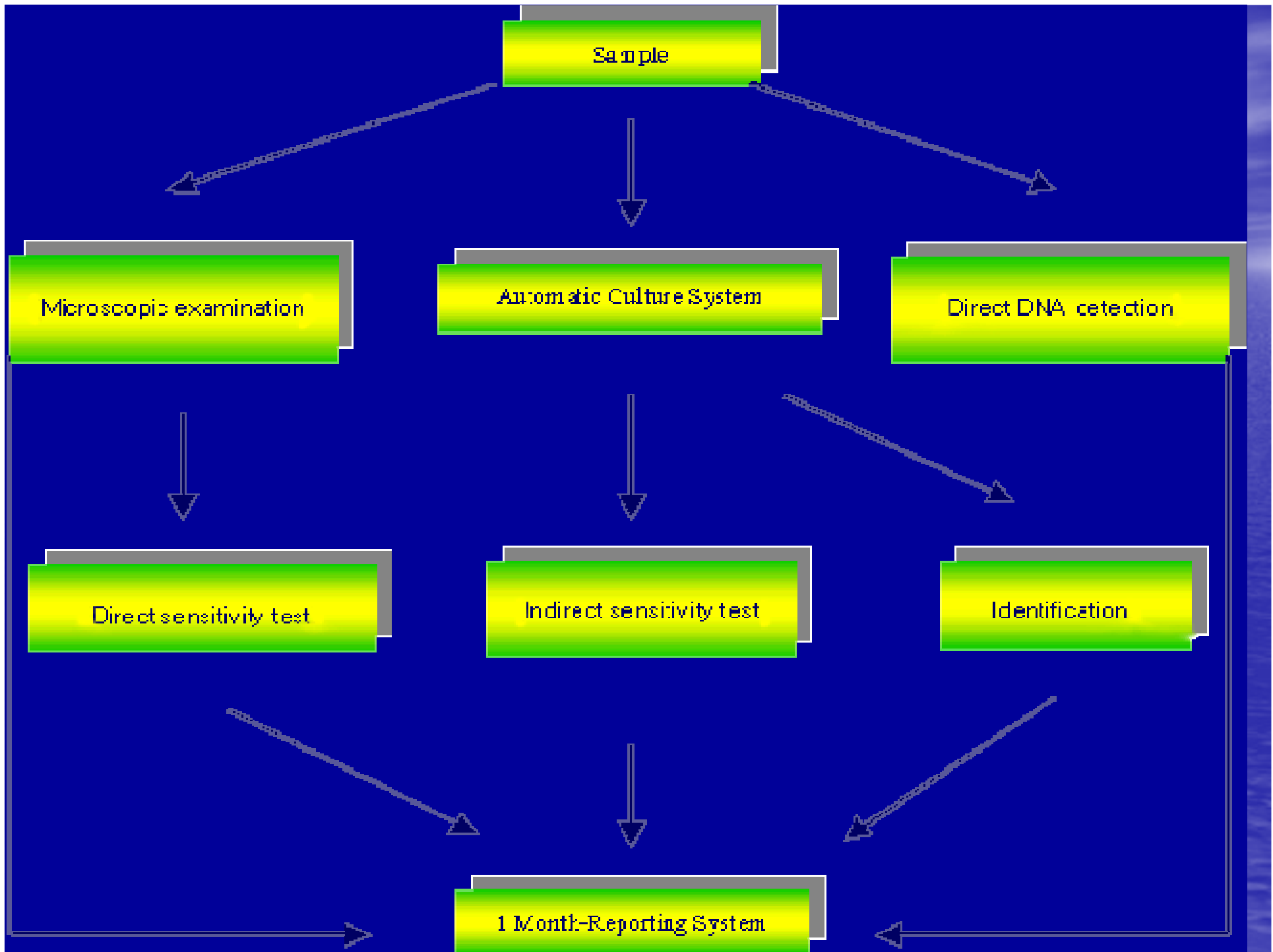


Positive Reaction: 18mm



TB infection vs. TB disease

	TB INFECTION	TB DISEASE (IN THE LUNGS)
M. tuberculosis	In the body	In the body
Skin test	Usually positive	Usually positive
Chest x-ray	Usually normal	Usually abnormal
Sputum smears & cultures	Negative	Positive
Symptoms	No	Yes. Cough, fever, weight loss, malaise, fatigue, night sweats
Infectious?	No	Often infectious before treatment
Case of TB?	No	Yes



New Diagnostic Tools

- **BACTEC TECH.**
- **PCR.**
- **RFLP.**

BACTEC

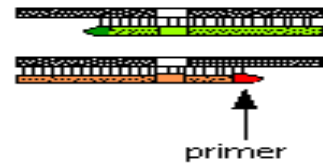
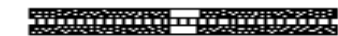
- A commercial system for detection of mycobacteria in samples. The basis of the system is growth of mycobacteria in liquid culture medium containing radioisotope-labeled palmitic acid.



Polymerase Chain Reaction

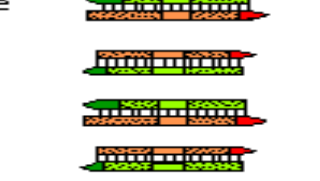
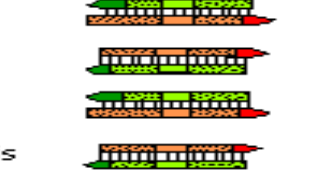
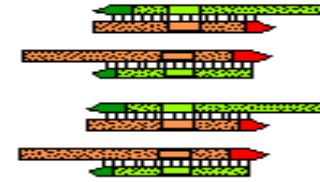
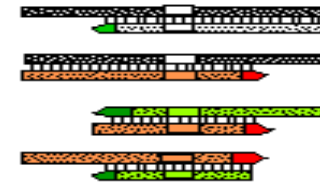
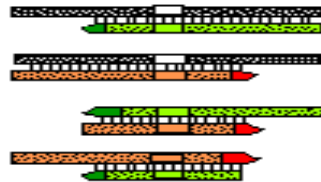
POLYMERASE CHAIN REACTION

DNA region of interest.



primer

1. DNA is denatured. Primers attach to each strand. A new DNA strand is synthesized behind primers on each template strand.



2. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

3. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

4. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

5. Continued rounds of amplification swiftly produce large numbers of identical fragments. Each fragment contains the DNA region of interest.

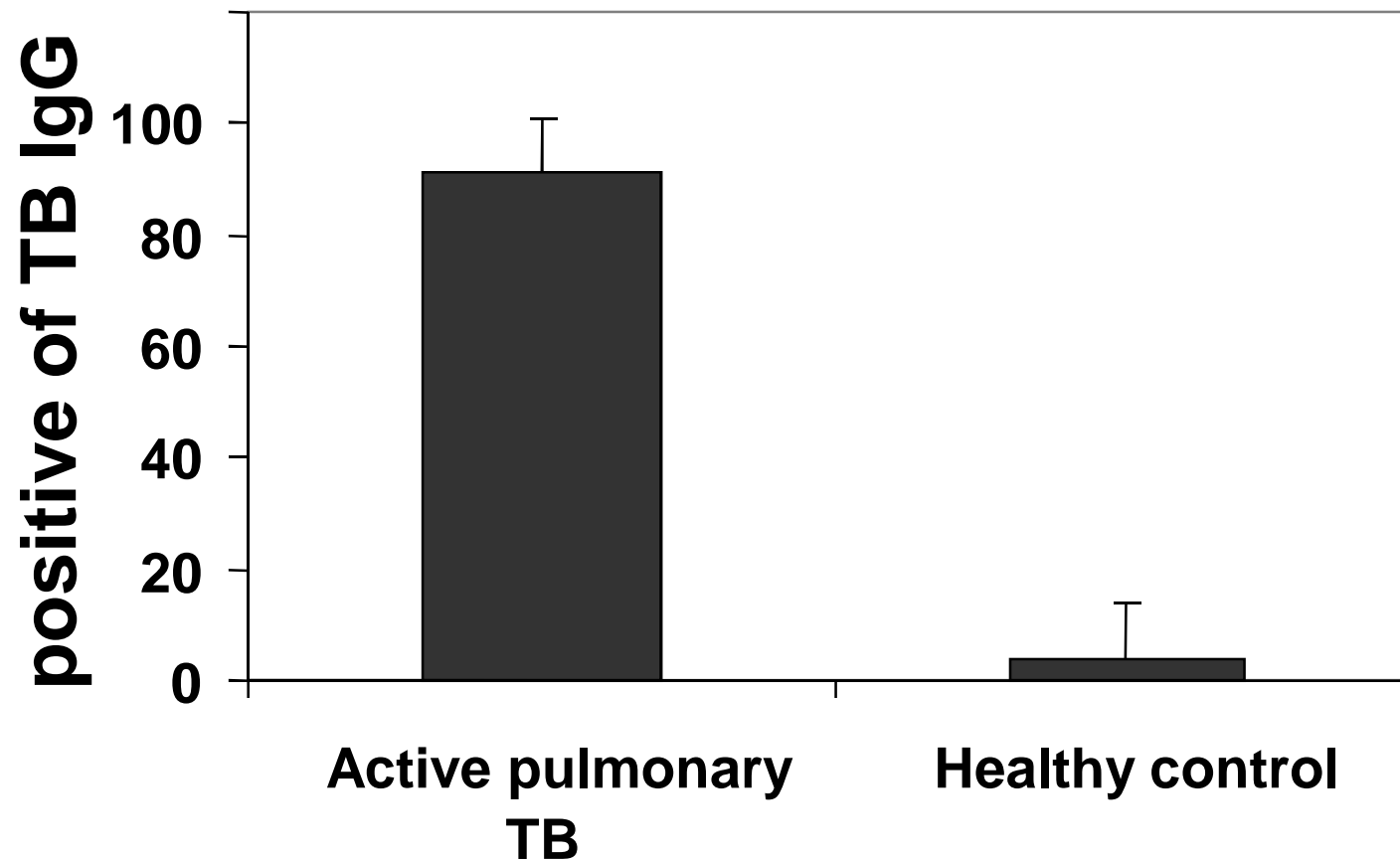
IMMUNODIAGNOSIS

OF TUBERCULOSIS:

AN UPDATE

**Serologic Diagnosis of
TB Using a Simple
Commercial
Multiantigen
Assay**

ELISA results from patients and healthy individuals



مقارنة طرق التشخيص المختلفة بطريقة الأليزا في المجموعة الفعالة للذئب ومجموعة الأصحاء

طرق التشخيصية	تعداد ونسبة مرضى الذئب	تعداد ونسبة الأصحاء
(Z-N) عينات السعال الإيجابية	60 (85.7%)	0 (0%)
المزرعة الإيجابية	48 (68.6%)	0 (0%)
الانسجة السنية	53 (75.7%)	0 (0%)
اختبار النيويركسين	50 (71.4%)	0 (0%)
اختبار الأليزا الإيجابي	61(87%)	3 (4%)
كمية الجسم المضاد بالطريقة المصلية	411±329 IU/ml	111±79 IU/ml

K. Weldingh, I. Rosenkrands, L. M. Okkels, T. M. Doherty, and P. Andersen

Assessing the Serodiagnostic Potential of 35 Mycobacterium tuberculosis Proteins and Identification of Four Novel Serological Antigens

J. Clin. Microbiol., January 1, 2005; 43(1): 57 - 65.

S. Banerjee, A. Nandyala, R. Podili, V. M. Katoch, K. J. R. Murthy, and S. Hasnain

Mycobacterium tuberculosis (Mtb) isocitrate dehydrogenases show strong B cell response and distinguish vaccinated controls from TB patients

PNAS, August 24, 2004; 101(34): 12652 - 12657.

[

**M. B. Conde, P. Suffys, J. R. Lapa e Silva,
A. L. Kritski, and S. E. Dorman**

**Immunoglobulin A (IgA) and IgG
Immune Responses against P-90
Antigen for Diagnosis of Pulmonary
Tuberculosis and Screening for
Mycobacterium tuberculosis Infection**

**Clin. Diagn. Lab. Immunol.,
January 1, 2004; 11(1): 94 - 97.**



Product	Unit	Cost (US\$)
4-FDC (R150/H75/Z400/E275)	Loose 1000 tabs	30.50
	Blisters 672 tabs	22.00
2-FDC (R150/H100)	Loose 1000 tabs	11.66
	Blisters 672 tabs	8.87
HE FDC (H150/E400)	Loose 1000 tabs	11.77
	Blisters 672 tabs	8.92
Streptomycin 0.75g	50 Vials	2.70
Isoniazid 300mg	Loose 1000 tabs	3.65
	Blisters 672 tabs	3.76
Ethambutol 400mg	Loose 1000 tabs	10.92
	Blisters 672 tabs	8.67
Pyrazinamide 400mg	Loose 1000 tabs	12.64
	Blisters 672 tabs	9.81

GDF Catalogue: www.stoptb.unwebbuy.org

“الوقاية خير

من العلاج”



For *my* sake
**DON'T
SPIT**

DISTRIBUTED BY YOUR TUBERCULOSIS ASSOCIATION—PAID FOR BY 

TUBERCULOSIS



YOUR KISS OF
AFFECTION
THE GERM OF
INFECTION

TOWN OF HEMPSTEAD, N.Y. - HEALTH OFFICER
U.S.P.H. FEDERAL AGENCY PROJECT - DISTRICT #

Cost per patient

- The average cost per patient is **\$10,873** (range, \$1,033-\$21,306).

- © 2006 (CDC)

EAT

THESE EVERY DAY



MILK - a pint for adults - more for children
cheese or evaporated milk or dried milk
ORANGES tomatoes grapefruit - raw cab-
bage or salad greens at least one of these
VEGETABLES green or yellow - some raw
some cooked **FRUITS** in season also dried
and canned fruit **BREAD** and cereal - whole
grain products or enriched white bread and
white flour **MEAT** poultry fish - dried beans
peas or nuts **EGGS** - 3 or 4 a week cooked any
way you choose or used in prepared dishes -
BUTTER vitamin rich fats and peanut butter
Then eat any other foods you may choose

BCG Vaccine

- ❖ يعطى للاطفال عند الولادة
- ❖ المخالطين لحالات الدرن موجبة القشع
- ❖ الاطفال الذين لم يطعموا من قبل
- ❖ عدم وجود ندبة خلال ٣ شهور من التطعيم ولديهم اختبار التبروكولين سلبي (أقل من ١٠ ملم)

Health Education

- ❖ **Importance:**
- ❖ **Society – Diabetic-
Malnutrition – Schools –
Contacts – Health team**
- ❖ **Patients have to continue
medication**

● رفع مستوى المعيشة مثل

تحسين التغذية والمسكن

وتهوية المنازل وتعريضها

للشمس وضوء النهار

يساعد على خفض انتشار

المرض

● **فإن المؤمن القوي خير
وأحب إلى الله من المؤمن
الضعيف**

● **وقال صلى الله عليه وسلم
"تداوا فإن الذي خلق الداء
خلق الدواء"**

THANK YOU

