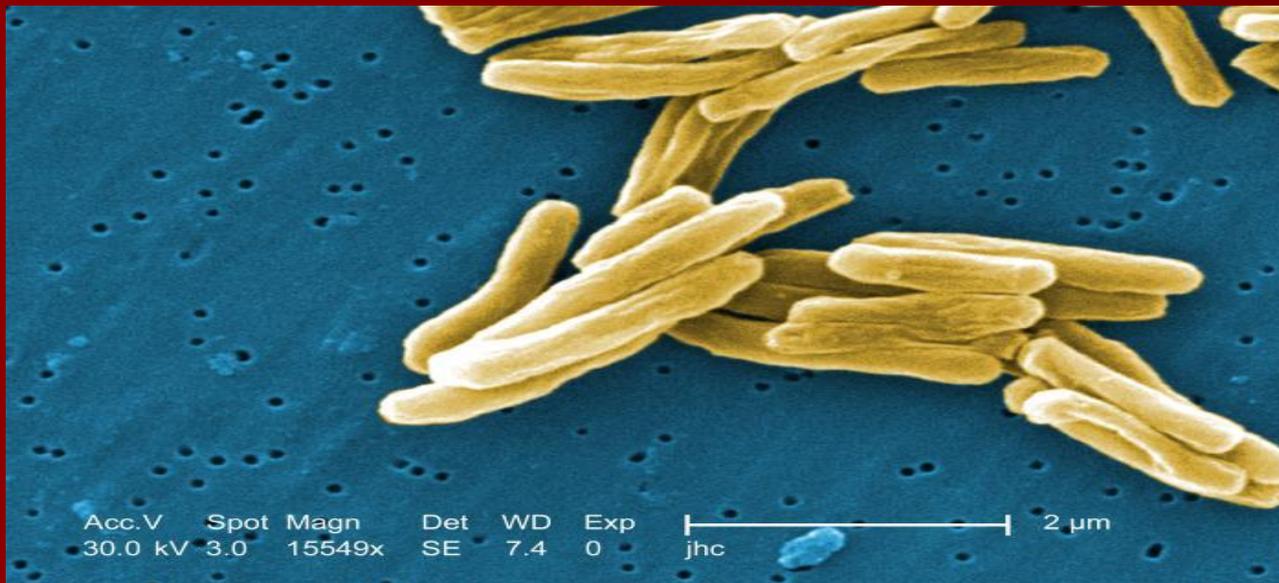


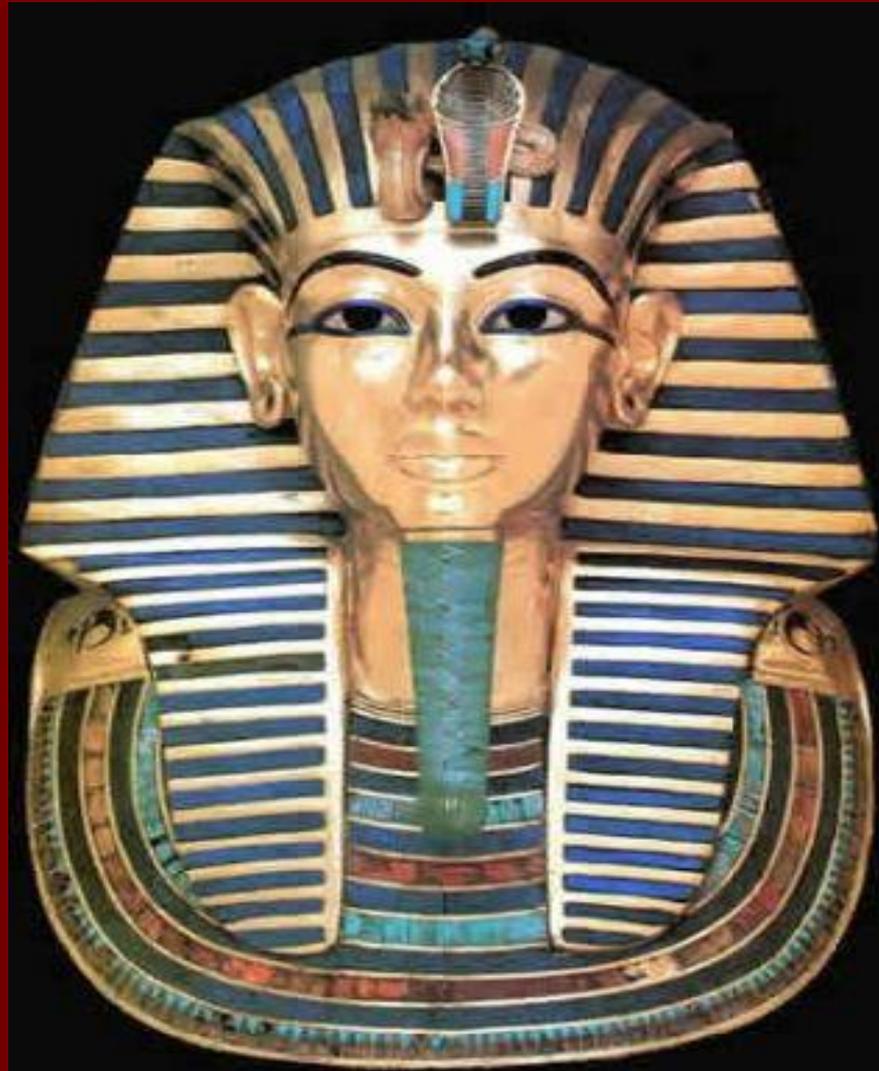
Update on Tuberculosis Laboratory Investigation



Adnan Al-Khatib MD
Al-Khatib Medical Laboratory

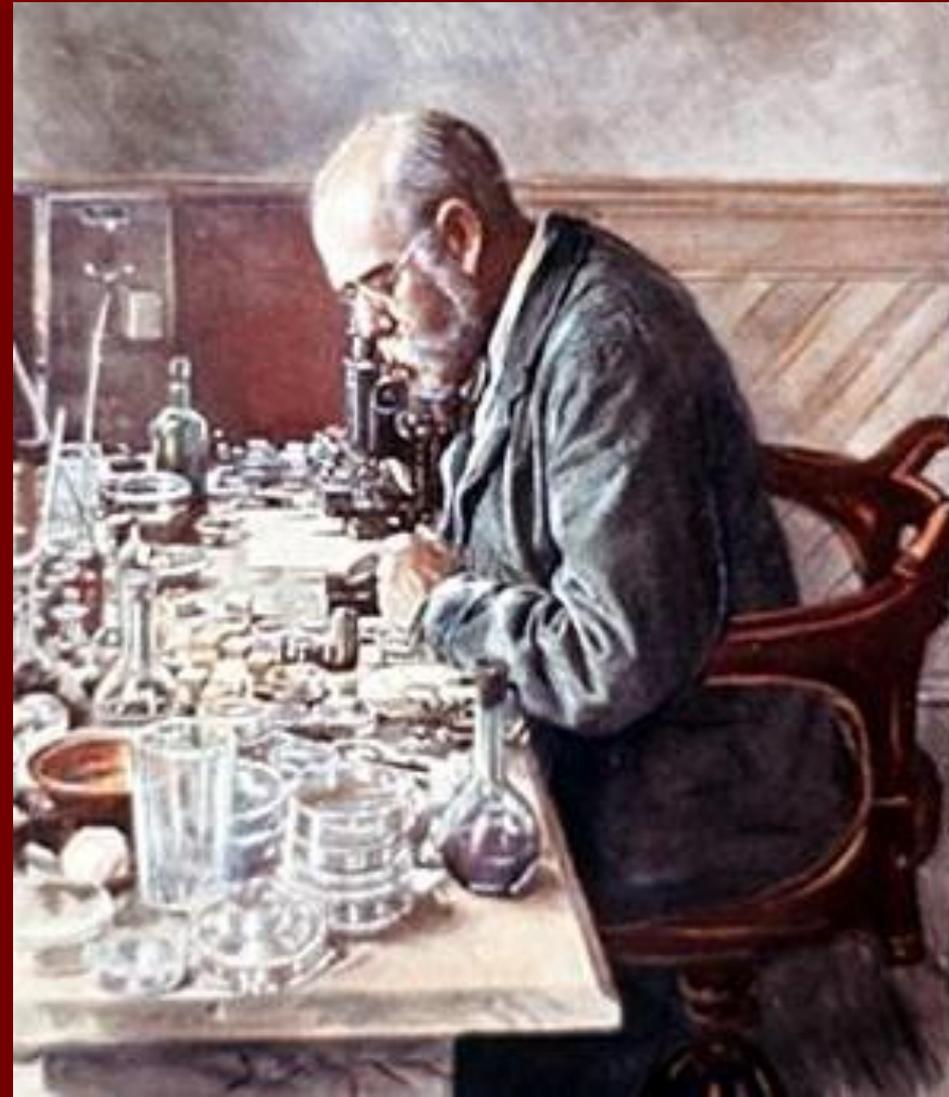
HISTORY of Tuberculosis

Tuberculosis Is an
Ancient Disease
Identified as Spinal
Tuberculosis in
Egyptian
Mummies History
dates to 1550 –
1080 BC
**Identified by
PCR**



A tribute to Robert Koch

- World TB Day is March 24. This annual event commemorates the date in 1882 when Dr. Robert Koch announced his discovery of *Mycobacterium tuberculosis*, the bacteria that cause tuberculosis (TB)



Some scientific achievements through 125 years

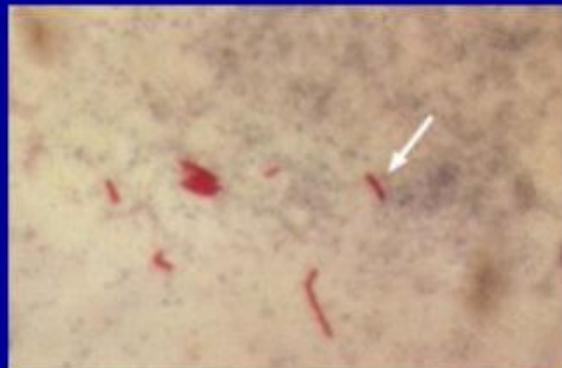
1882
R. Koch,
M. tuberculosis



1920
Löwenstein,
Jensen



1882/1883
Ehrlich,
Ziehl and
Neelsen



1921
Calmette
Guerin



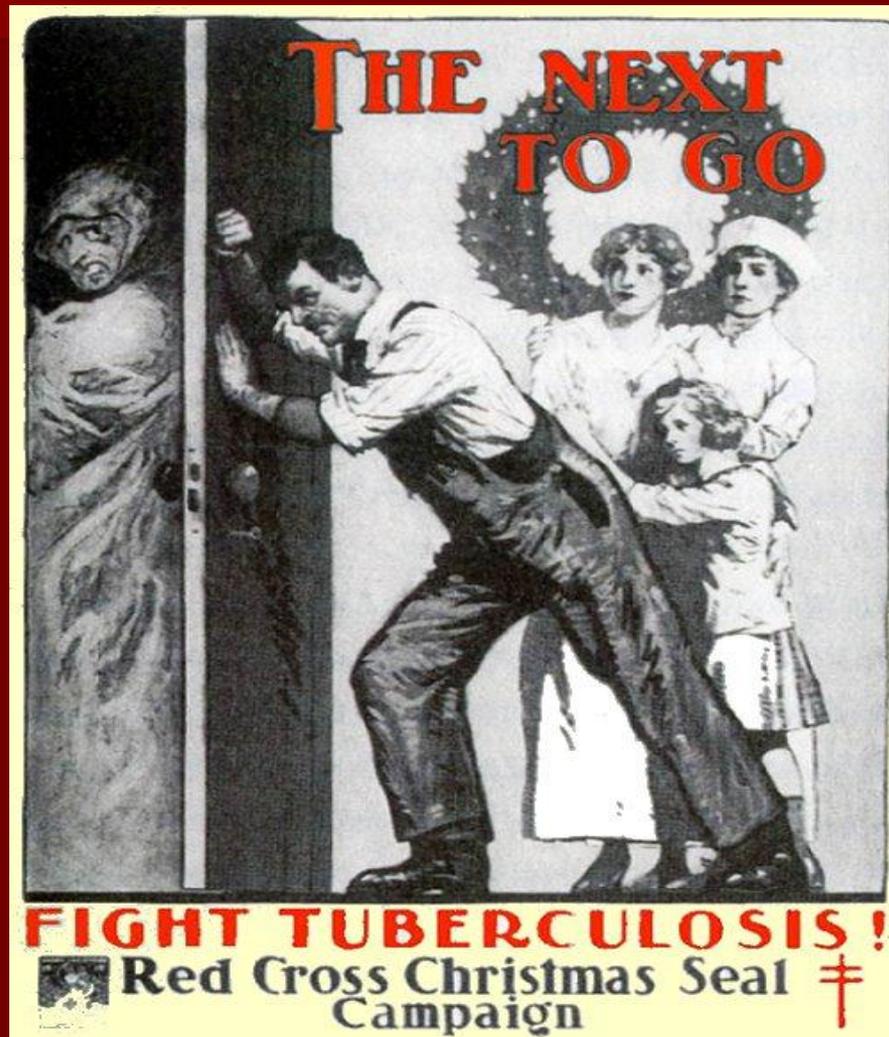
1907/1909
von Pirquet,
Mantoux
TST



1943
Waksman,
Streptomycin



Tuberculosis A Historical Disease Progress to Future



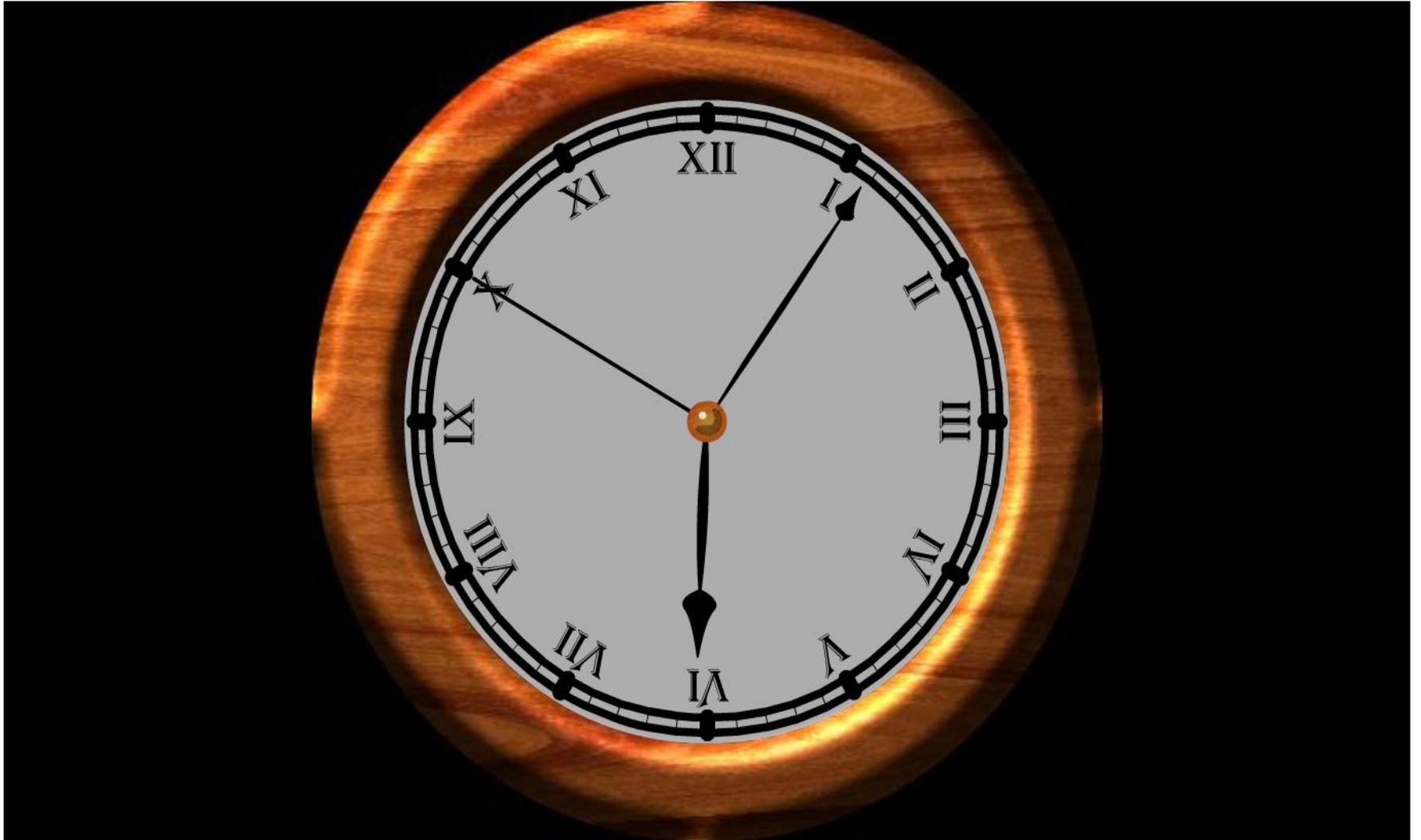
IT IS ESTIMATED THAT BETWEEN 2002 AND 2030, APPROXIMATELY 1000 MILLION PEOPLE WILL BE NEWLY INFECTED, OVER 100 MILLION PEOPLE WILL GET RICH, AND 20 MILLION WILL DIE OF TB ... WORLD HEALTH ORGANIZATION



THE RETURN OF THE WHITE PLAGUE

GLOBAL POVERTY AND THE 'NEW' TUBERCULOSIS
EDITED BY MATTHEW GANDY AND ALIMUDDIN ZUMLA

Global incidence of tuberculosis
Still rising as a result of the growing epidemic in Africa



Laboratory diagnosis

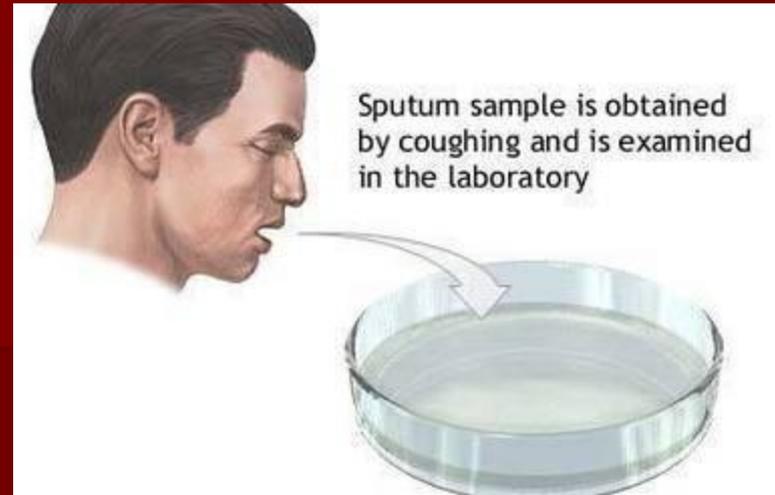
- Direct Microscopy
- Culture
- Nucleic acid technology
- Immunological methods
 - Skin test (TST)
 - Rapid test
 - IGRA test

Microscopy and Culturing still a top priority

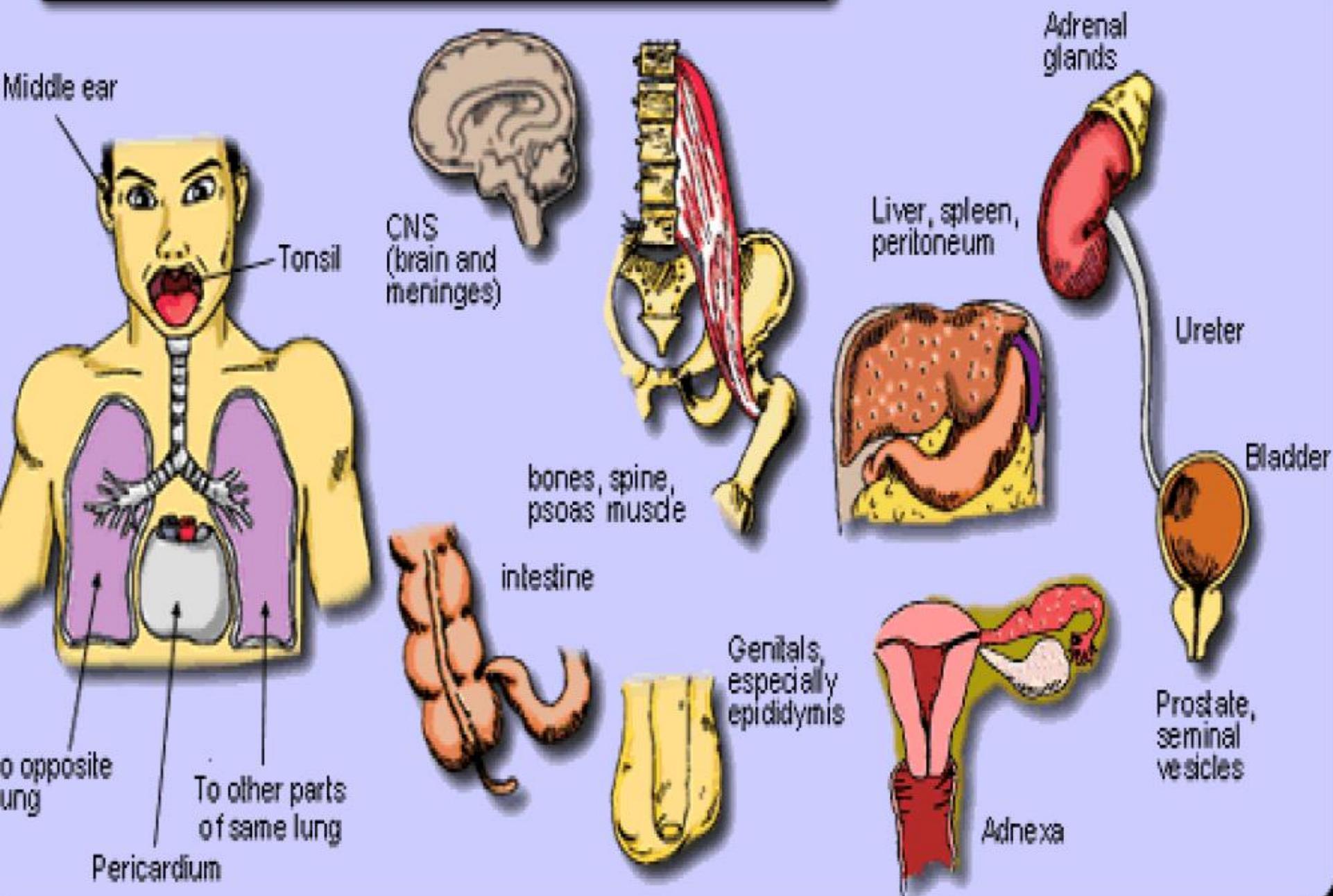


Pulmonary Tuberculosis

Morning Sputum specimens



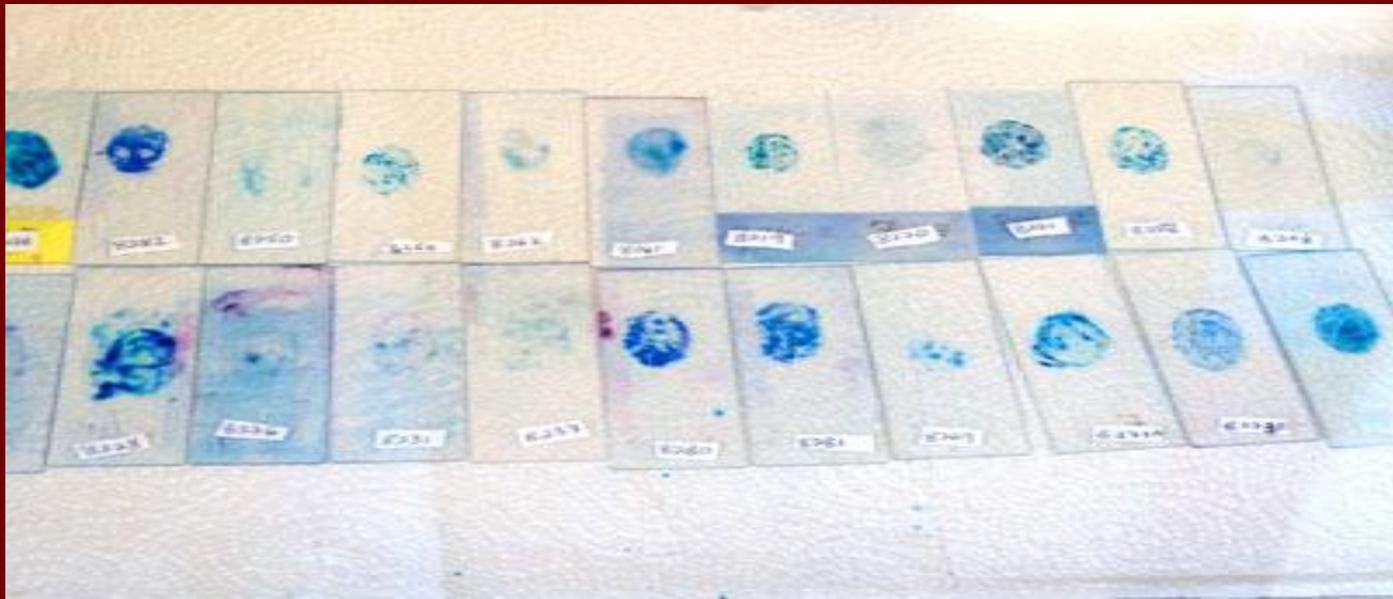
Tuberculosis Affects Many Parts of the Body



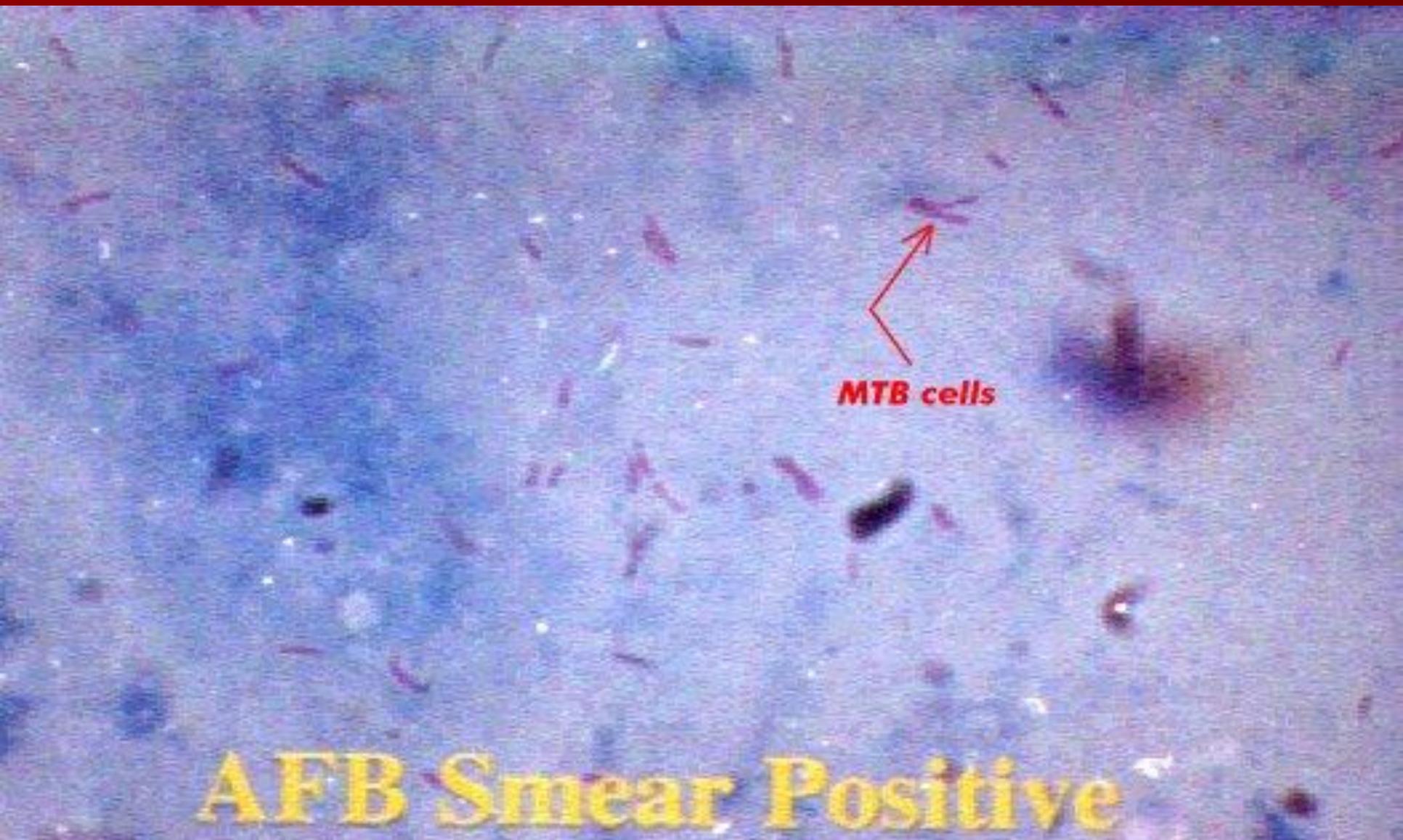
Laboratory Diagnosis

1- Sputum smears stained by Z-N stain

Two morning successive mucopurulent sputum samples are needed to diagnoses pulmonary TB.



Smear showing Acid Fast Bacilli.



MTB cells

AFB Smear Positive

What is Smear Positivity WHO

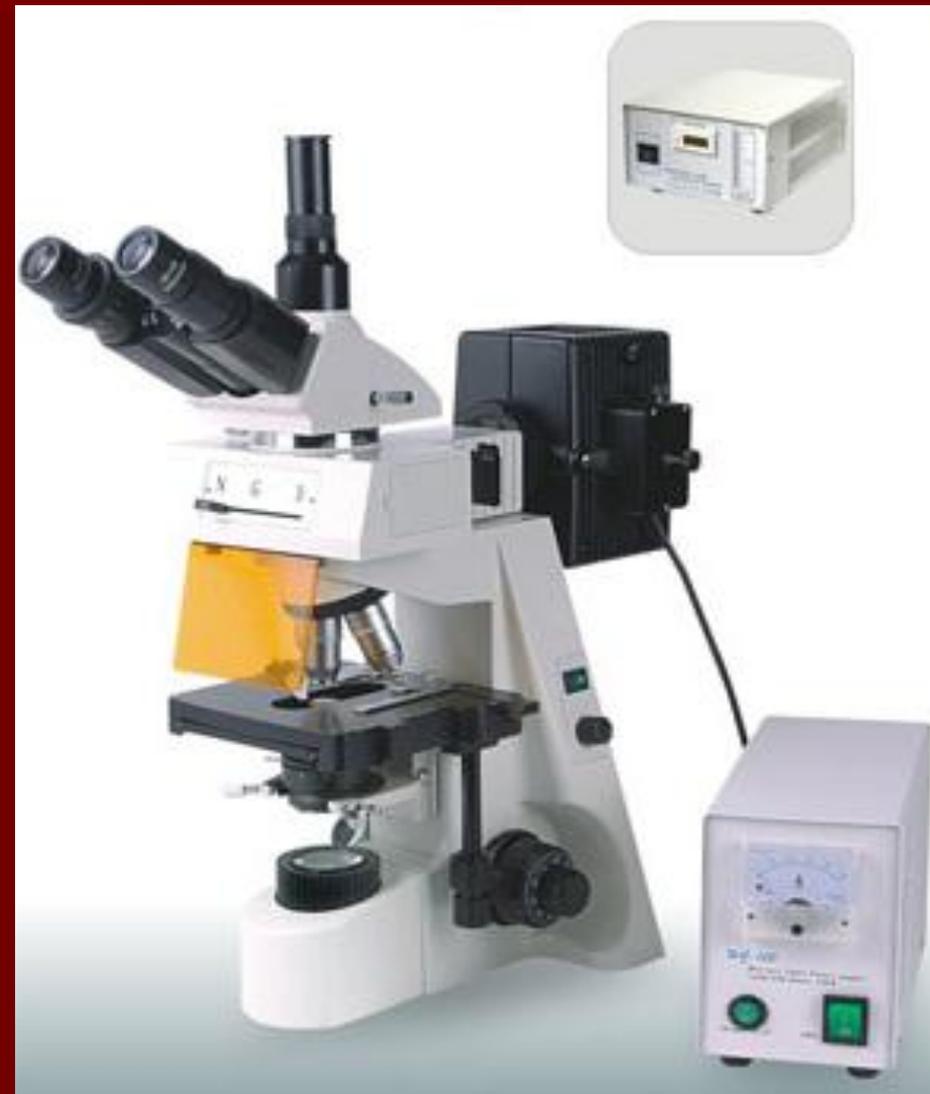
All patients who have submitted two
Specimens and found to be positive
for identification of AFB

Grading

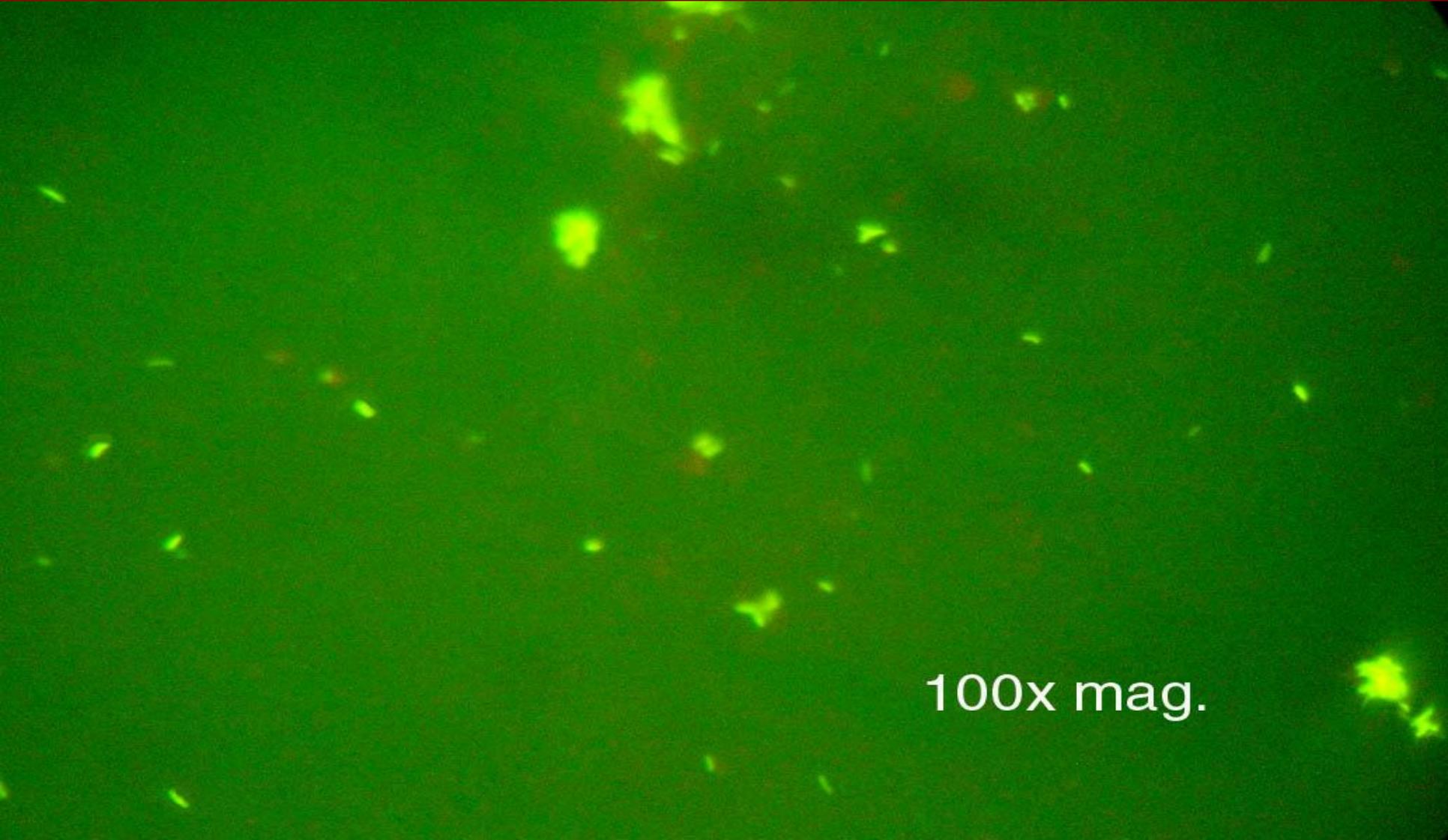
If the slide has	Result	Grading	No. of fields to be examined
More than 10 AFB /oil immersion field	pos	3+	20
1-10 AFB/ oil immersion field	pos	2+	50
10-99 AFB/100 oil immersion field	pos	1+	100
1-9 AFB/100 oil immersion field	pos	Scanty*	100
No AFB in 100 oil immersion field	neg		100
*record actual number of bacilli seen in 100 fields			

2- Detecting AFB by fluorochrome stain using fluorescence microscopy:

The smear may be stained by auramine-O dye.



Acid Fast Bacilli as seen under Fluorescent Microscope



100x mag.

Direct Microscopy

- Preferred to do two methods
- Make sure it is real sputum
- At least 3 specimen

Microscopic Exam

Smears:

Specificity 42%

Sensitivity:

46% in pulmonary TB

31% in extrapulmonary TB

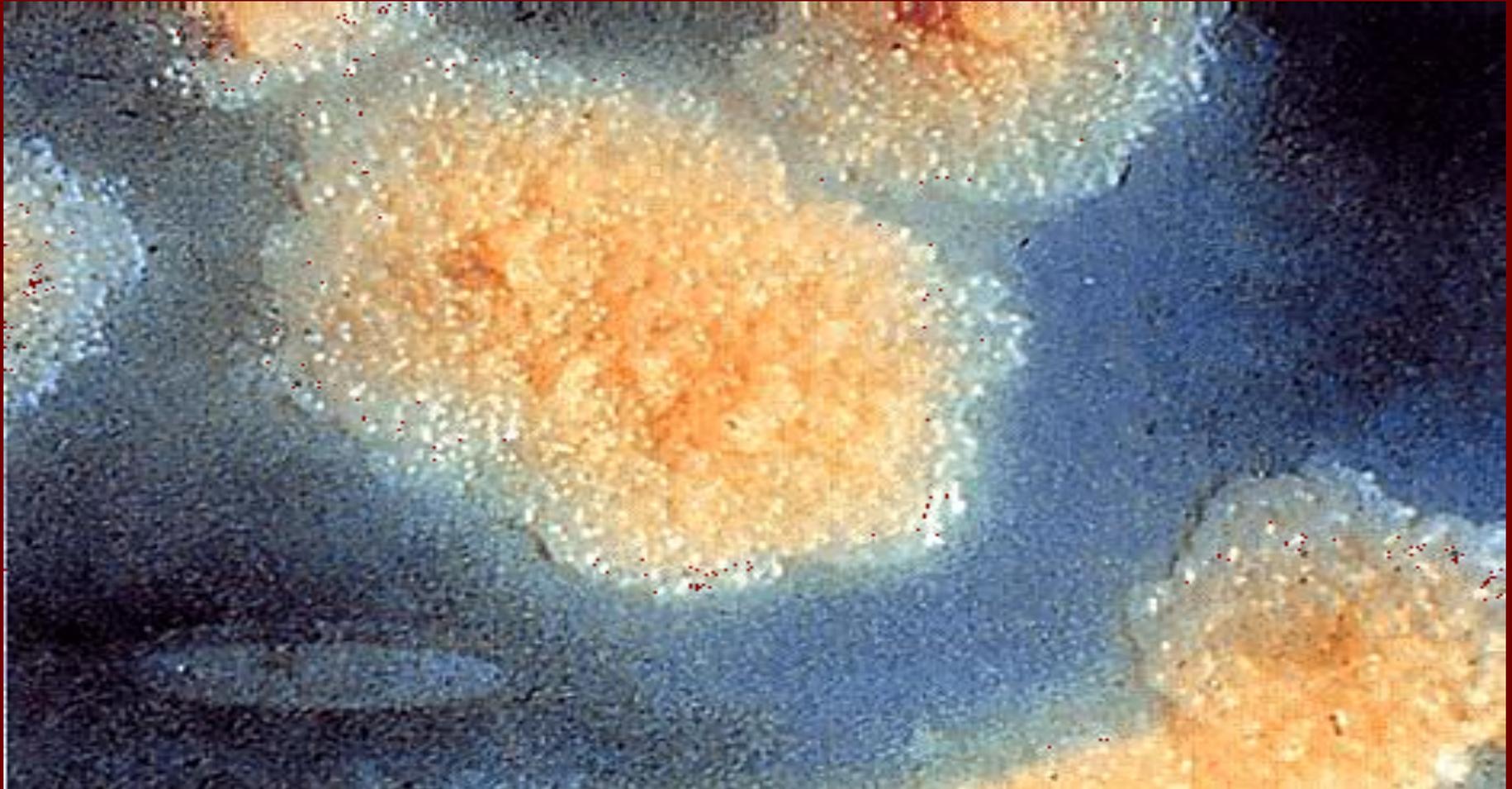
Culturing Mycobacterium

- Culturing for isolation of Mycobacterium spp continues to be a **Gold standard**, particularly in Developing countries.
- Need only 10 – 100 bacilli / 1 ml of sputum.

Growth of Acid fast bacilli on L J Medium.



Eight Week Growth of Mycobacterium tuberculosis on Lowenstein-Jensen Agar



Liquid culture

June 2007: Liquid culture and DST in LMIC were **endorsed by WHO's Strategic and Technical Advisory Group for TB (STAG TB)** and subsequently issued as **WHO policy**.

(see <http://www.who.int/tb/dots/laboratory/policy/en/index3.html>)



BacT/ALERT



MGIT

Culture

- Specificity 59%
- Sensitivity:
 - 63% in pulmonary TB
 - 42% in extrapulmonary

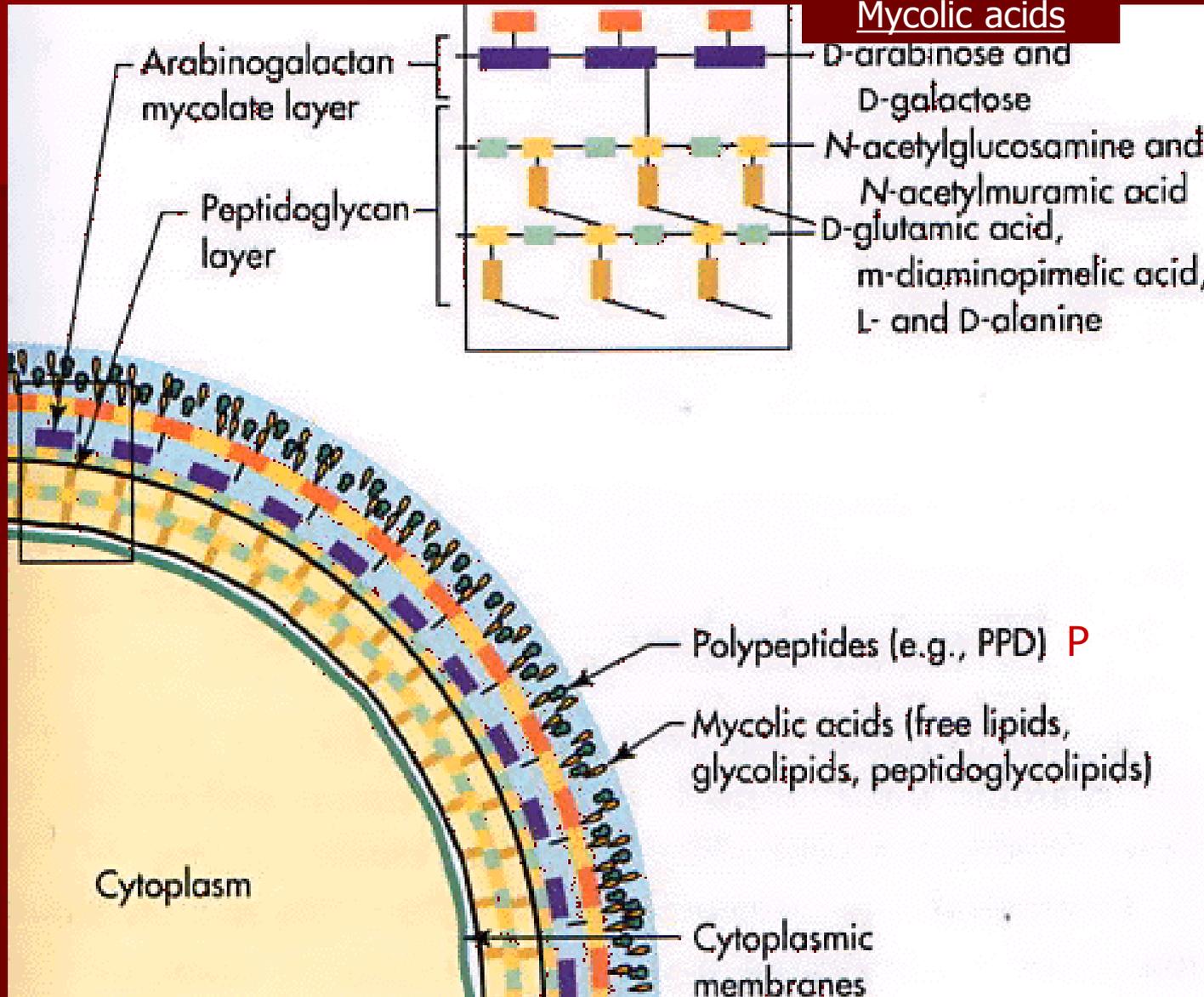
Limitation in Culturing

- Mycobacterium spp are slow growing.
- Need 6 – 8 weeks for growing.
- Specimens can be contaminated while growing, needs repeated specimens, in turn patients loose confidence in Laboratories.

Molecular Methods in Diagnosis of Tuberculosis



Lipid-Rich Cell Wall of Mycobacterium



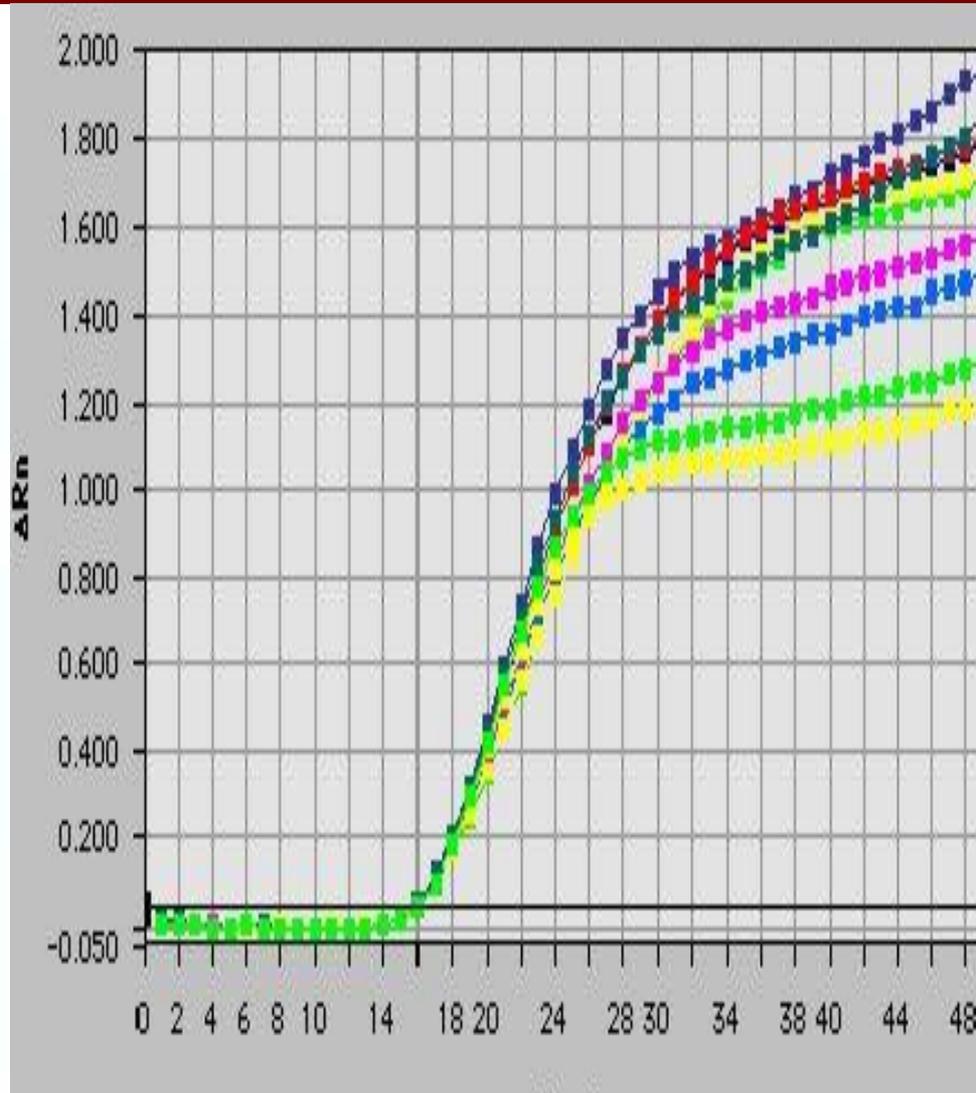
Protein Derivative)

III Polymerase Chain Reaction (PCR) & Gene probe

Advantages:

- Rapid procedure
(3 – 4 hours)
- High sensitivity (1-10
bacilli / ml sputum)

Real Time PCR replacing older Methods



**Mumtaz Ahmad Khan, Sajjad Hussain Mirza, Shahid Ahmed
Abbasi, Tariq Butt, Masood Anwar**

**PERIPHERAL BLOOD-BASED POLYMERASE CHAIN REACTION IN
DIAGNOSIS OF PULMONARY TUBERCULOSIS**

• **PCR PB**

Sensitivity 20%

Specificity 95%

PCR Site of infection

sensitivity 90%

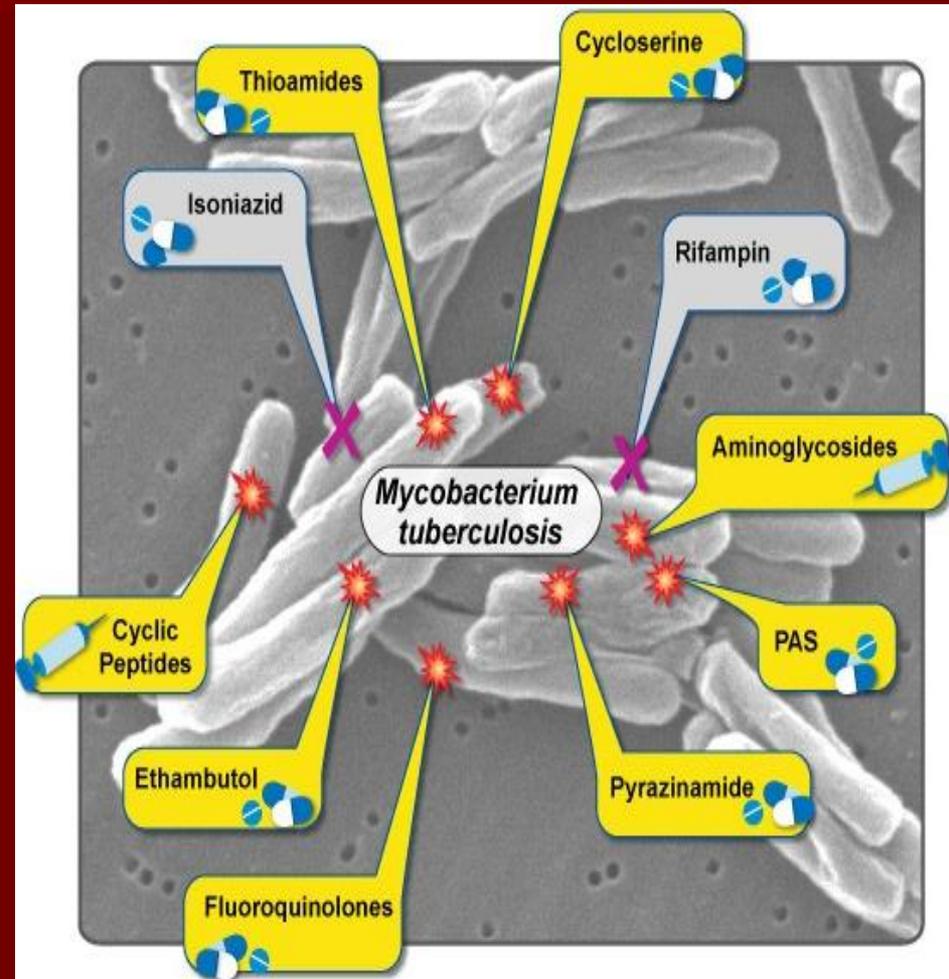
specificity 95%

Sensitivity for TB Detection

Technique	No. Bacilli	
Microscopy	5000-100000	Bacilli/ml
Culture	10 - 100	Bacilli/ml
PCR	1 - 10	Bacilli/ml

MDR Tuberculosis

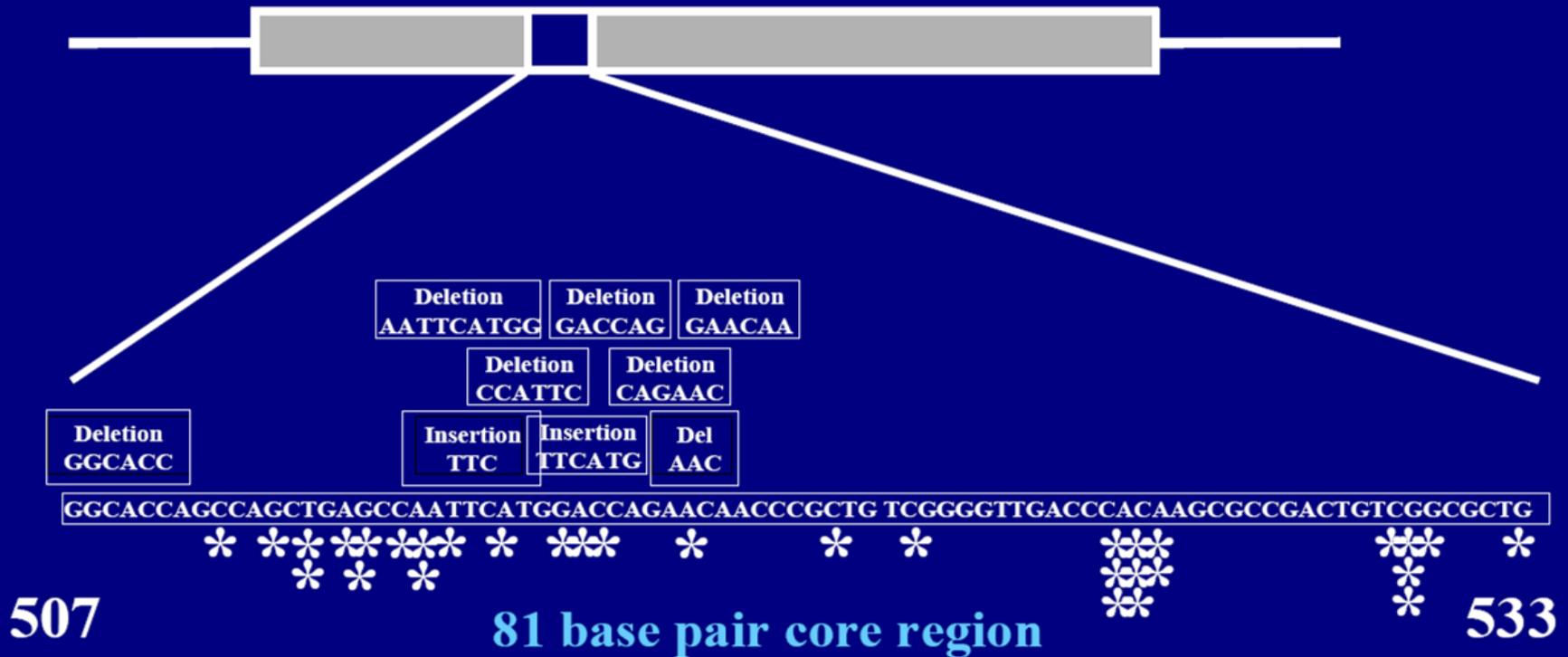
- **MDRTB** refers to strains of the bacterium which are proven in a laboratory to be resistant to the two most active anti-TB drugs, isoniazid and rifampicin. Treatment of MDRTB is extremely expensive, toxic, arduous, and often unsuccessful.



X Bacteria are resistant to this drug

Genetics of Rifampin Resistance in *M. tuberculosis*

rpoB



RIF resistance as surrogate marker for MDR TB

- RIF resistance as a mono resistance not very frequent (5-15% of them)
- 80-95% of RIF resistant strains are also resistant to INH



GeneXpert

**Automated Sample Prep,
Amplification and Detection**

<120 minutes

First Published: 2010-12-08

اختبار جديد قد يحدث ثورة في مكافحة السل

الاختبار الجديد يمكن من تشخيص المرض خلال 100 دقيقة،
في حين تستغرق الاختبارات الحالية 3 أشهر.



ميدل ايست أونلاين

لندن – من كيت كيلاند

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812

SEPTEMBER 9, 2010

VOL. 363 NO. 11

Rapid Molecular Detection of Tuberculosis and Rifampin Resistance

Catharina C. Boehme, M.D., Pamela Nabeta, M.D., Doris Hillemann, Ph.D., Mark P. Nicol, Ph.D., Shubhada Shenai, Ph.D., Fiorella Krapp, M.D., Jenny Allen, B.Tech., Rasim Tahirli, M.D., Robert Blakemore, B.S., Roxana Rustomjee, M.D., Ph.D., Ana Milovic, M.S., Martin Jones, Ph.D., Sean M. O'Brien, Ph.D., David H. Persing, M.D., Ph.D., Sabine Ruesch-Gerdes, M.D., Eduardo Gotuzzo, M.D., Camilla Rodrigues, M.D., David Alland, M.D., and Mark D. Perkins, M.D.

sample processing in 1730 patients with suspected drug-sensitive or multidrug-resistant pulmonary tuberculosis. Eligible patients in Peru, Azerbaijan, South Africa, and

CONCLUSIONS

The MTB/RIF test provided sensitive detection of tuberculosis and rifampin resistance directly from untreated sputum in less than 2 hours with minimal hands-on time. (Funded by the Foundation for Innovative New Diagnostics.)



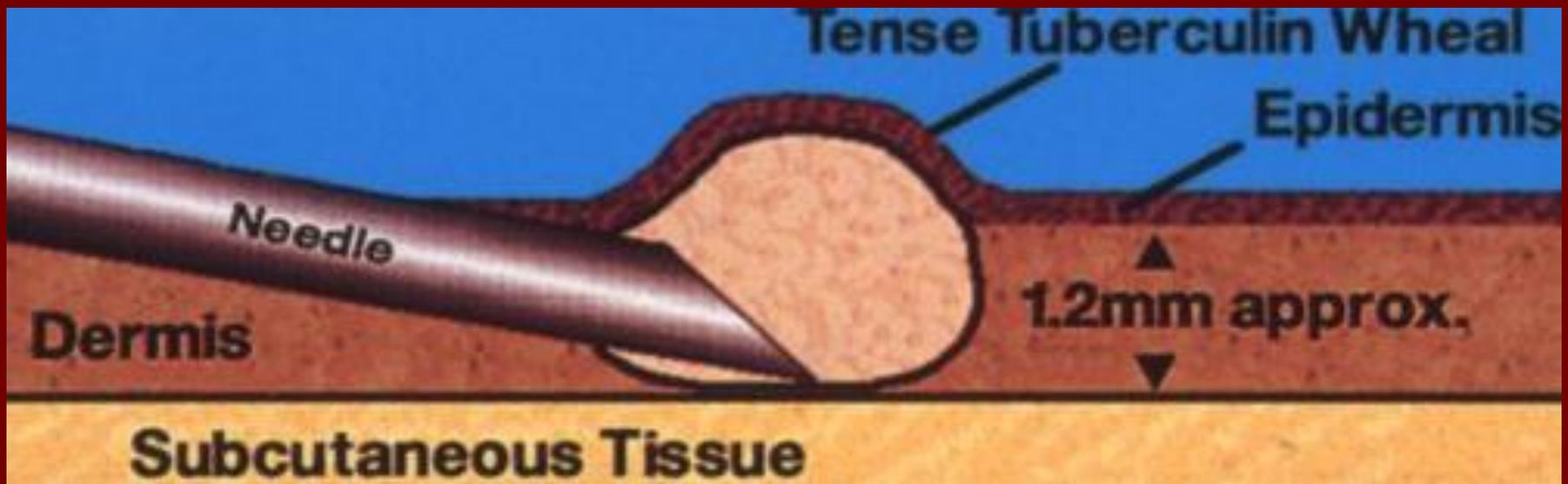
Immunological methods

- Tuberculin Skin test (TST)(Mantoux)
- Antibodies detections
- IGRA

Tuberculin skin test

- 1. Mantoux test
 - . 0.1 ml of PPD containing 5 TU is injected intradermally on flexor aspect of forearm
 - . Examine after 48 – 72 hrs
- Color change without induration is **not** included in the measurement

Applying the tuberculin skin test



Applying the tuberculin skin test



Courtesy of Dr. Marc Steben

Tuberculin Test (Mantoux Test)

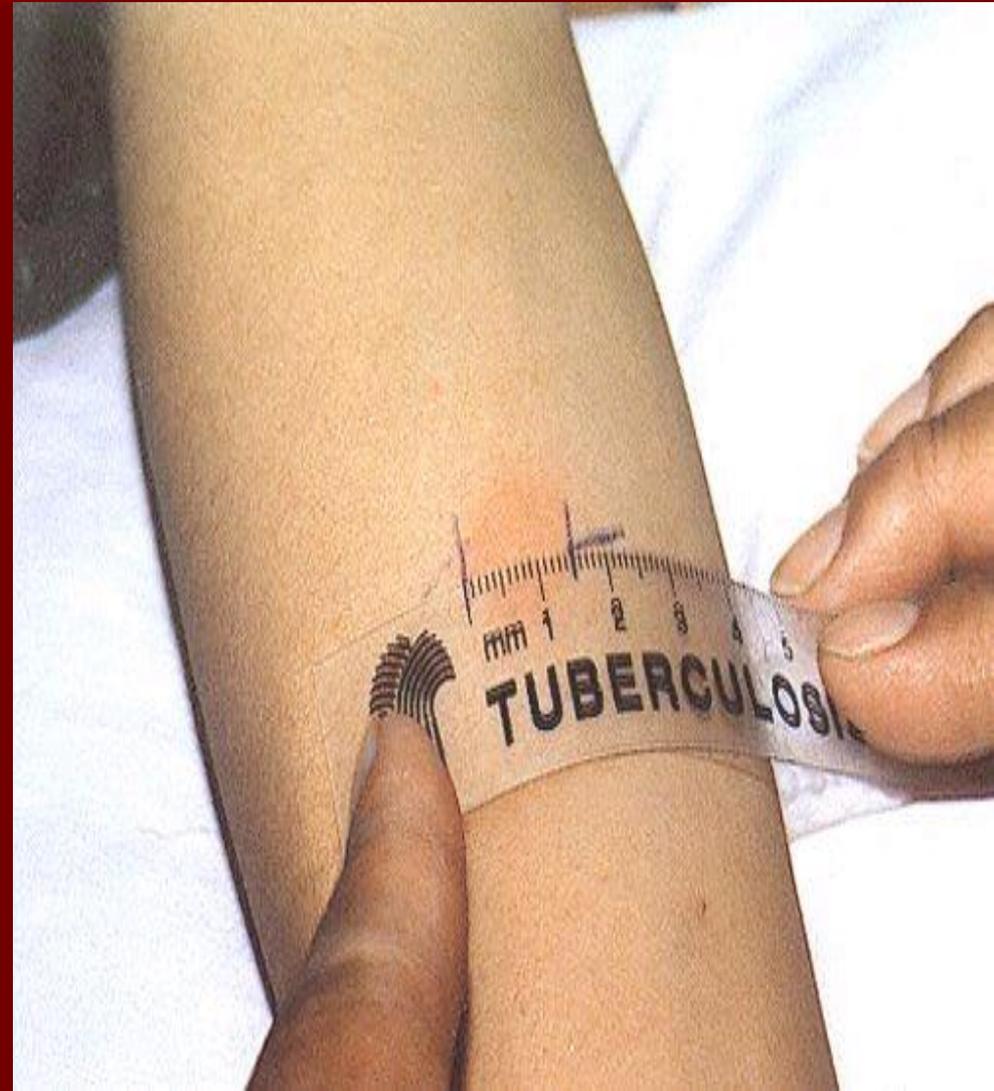
- Diameter of induration

Negative 0 - <5 mm

Positive >5 –15 mm

Strong Positive > 15 mm

- >10 mm children <4 years



Tuberculin skin test

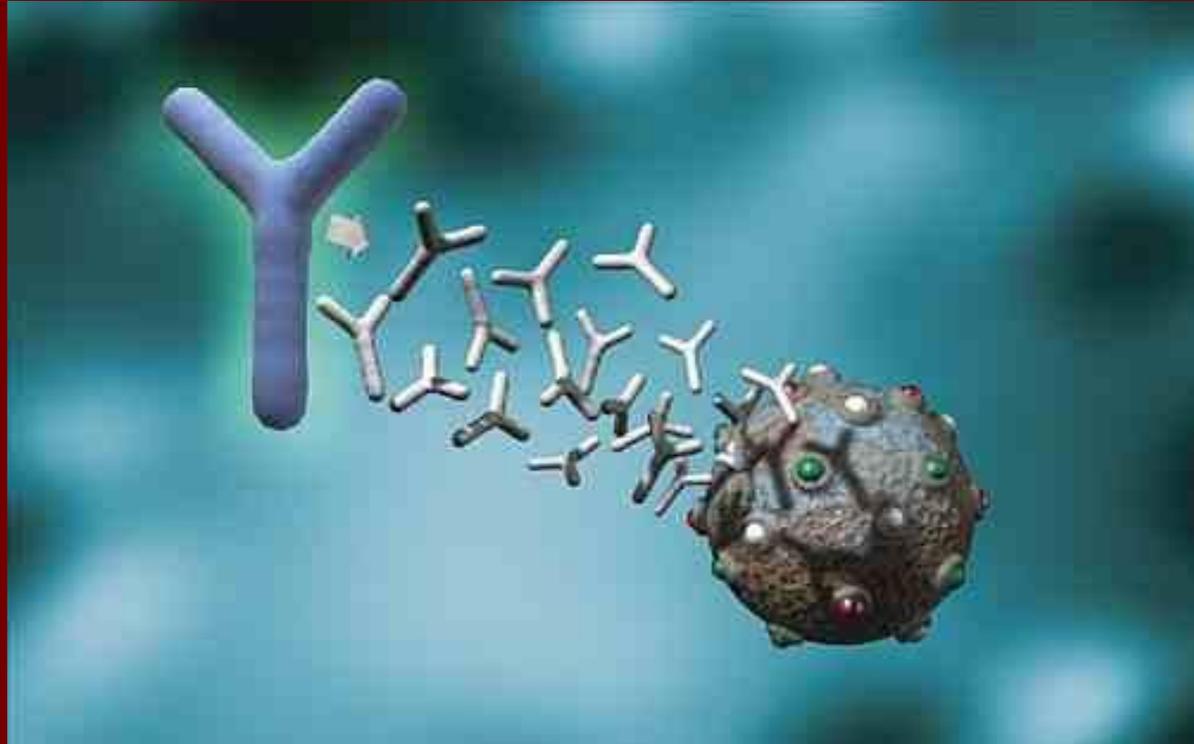
Specificity: 35.4% (cutoff: 10-mm induration).
Sensitivity: 65.8%.

Specific Detection of Tuberculosis Infection

Gerald H. Mazurek and Izuo Tsuyuguchi

*American Journal of Respiratory and Critical Care
Medicine* Vol 170. pp. 59-64, (2004)

Antibodies Detection



Nisar Khan, Ihsanullah Mian,* Zia-Ullah,** Jan Muhammad
**PERFORMANCE OF ICT-TB TEST IN THE DETECTION OF
PULMONARY AND EXTRA-PULMONARY TUBERCULOSIS**

JAMC 21(3): Jul-Sep 2009

The sensitivity, specificity, and negative predictive value of the ICT Tuberculosis test for pulmonary TB were 33.3%, 100%, and 52.9%





World Health Organization

Laboratory-based evaluation of 19 commercially available rapid diagnostic tests for tuberculosis

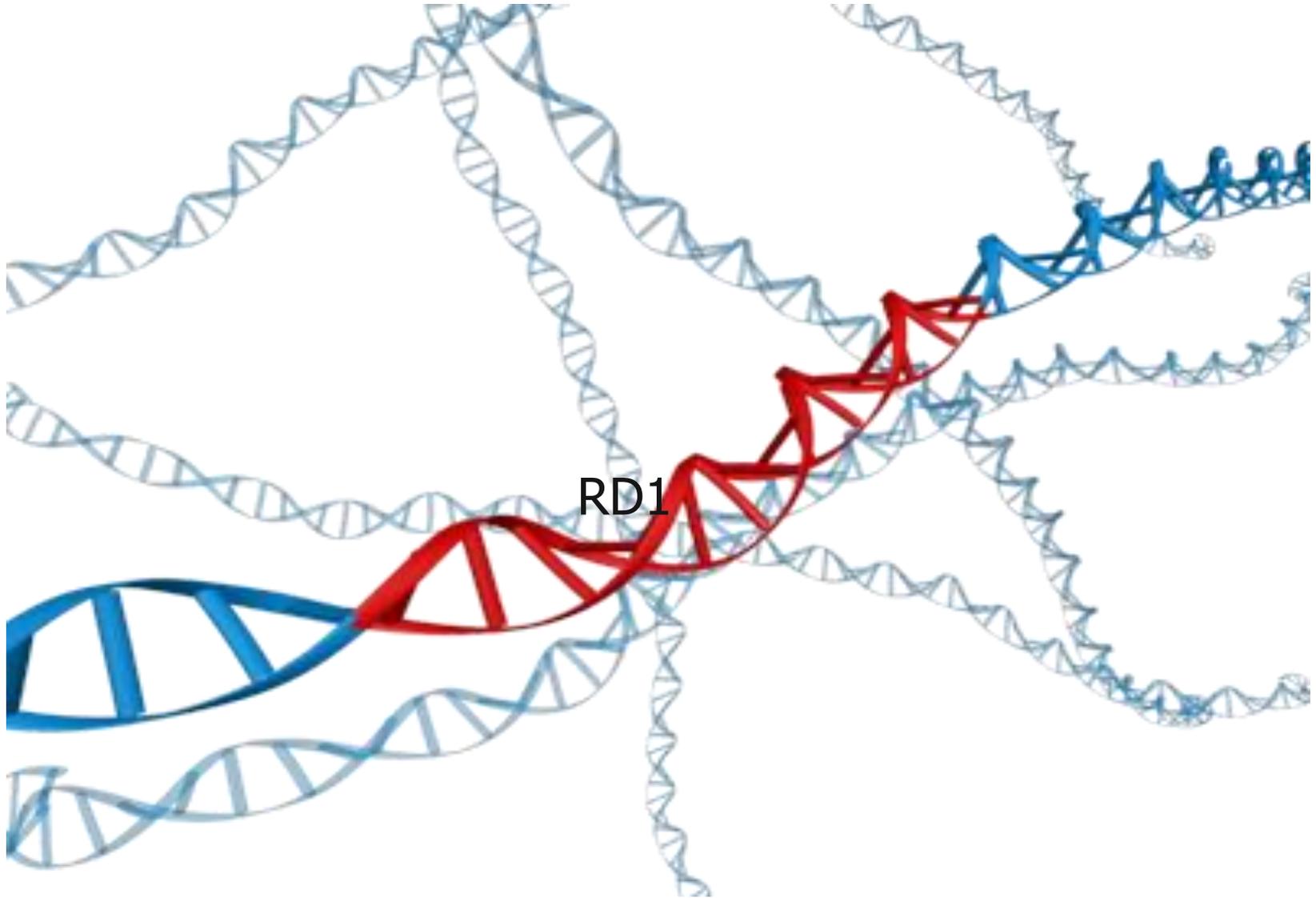
Currently marketed rapid serologic TB tests vary widely in performance but generally perform poorly compared to a combined reference standard using well-characterized archived serum specimens

Interferon γ Assay (IGRA)

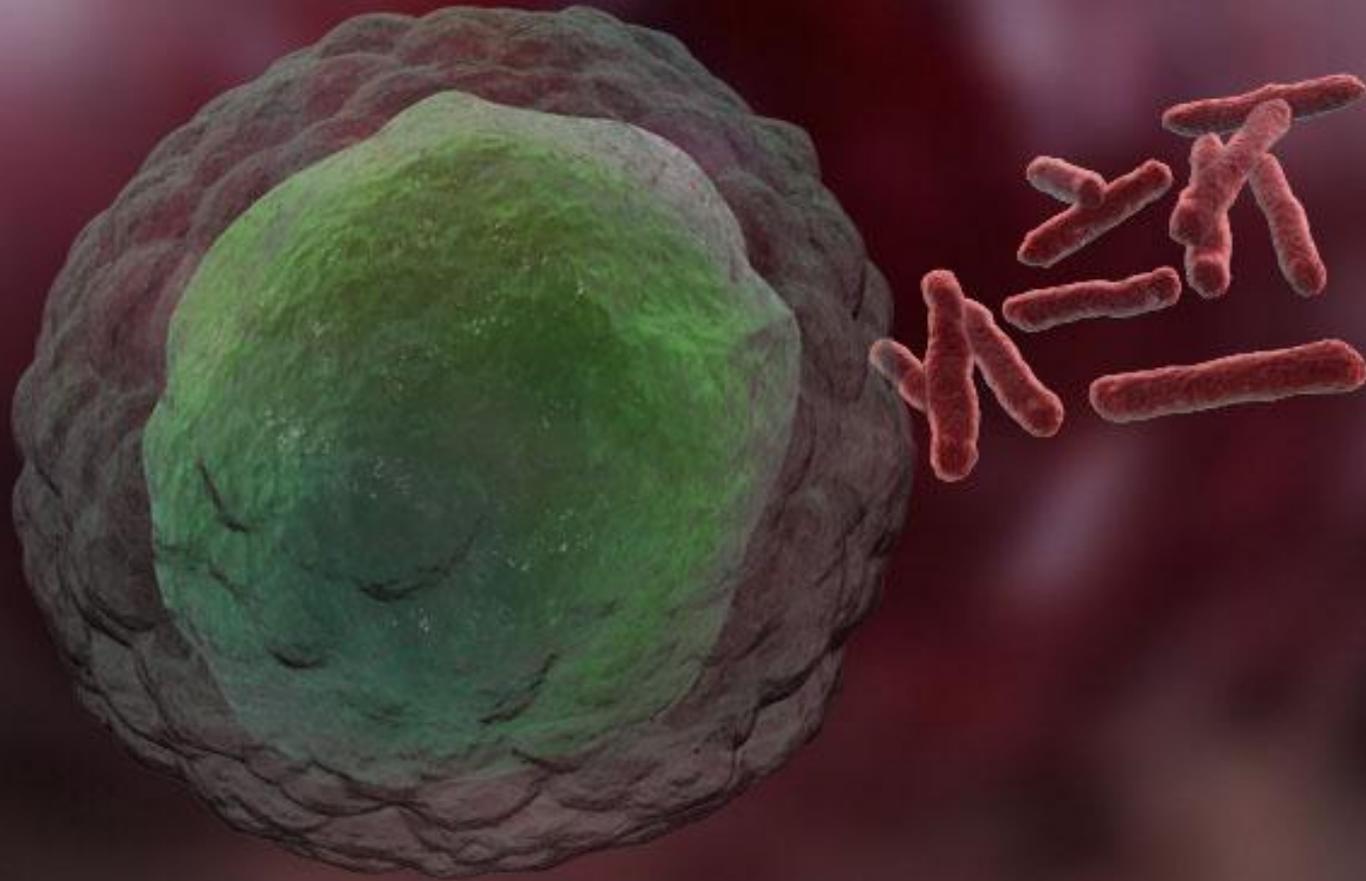
- QuantiFERON TB gold Test
- T-Spot Test.

whole-blood assays

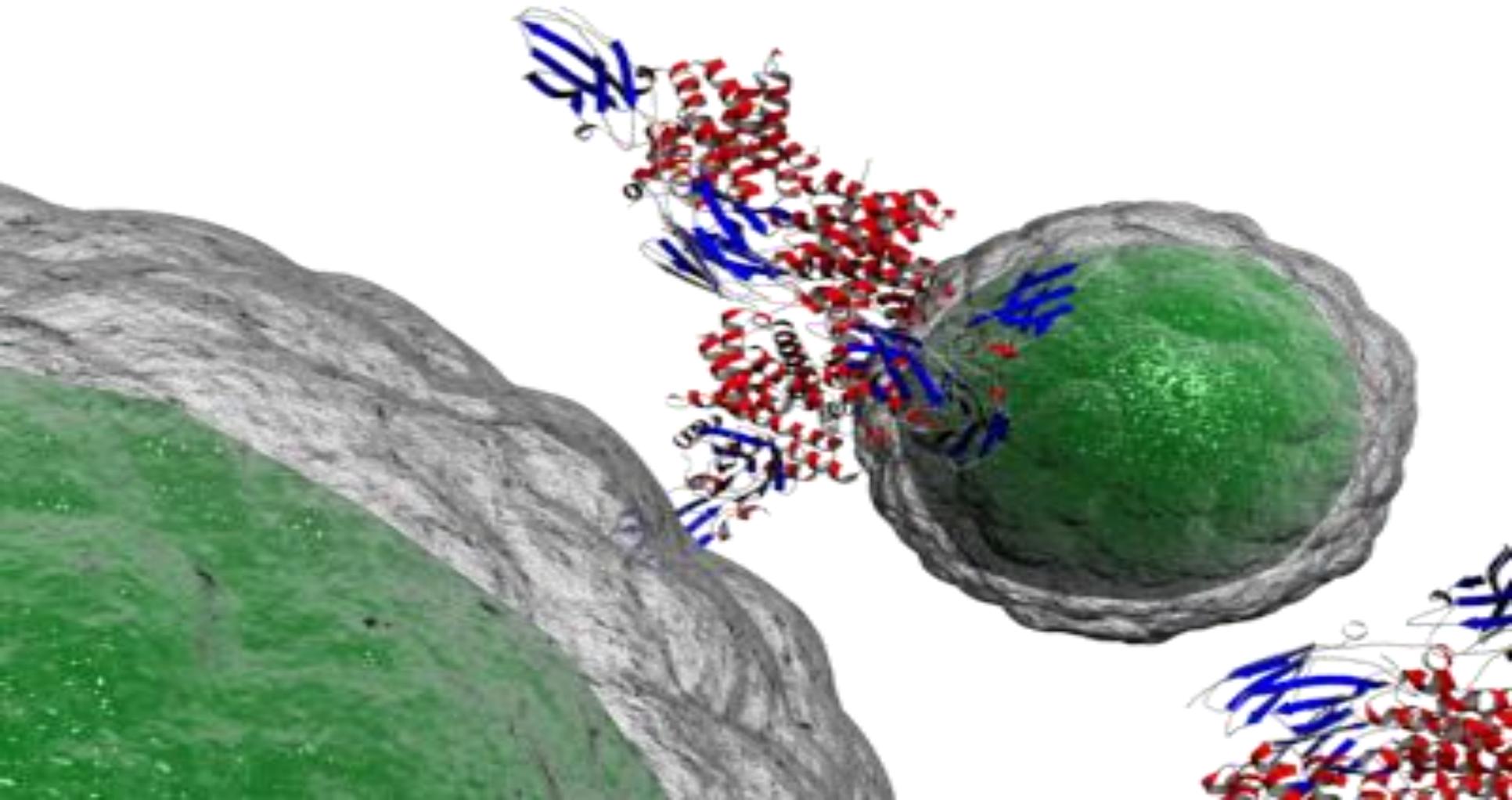
Mycobacterium DNA



ESAT-6
CFP-10



Interferon γ Released



IGRA

Specificity: 98.1%.

Sensitivity: 89%.

Specific Detection of Tuberculosis Infection An Interferon γ -based Assay Using New Antigens

*American Journal of Respiratory and Critical Care
Medicine* Vol 170. pp. 59-64, (2004)

- Effective in children less than 6 months of age.

IGRA positive result cannot distinguish between active tuberculosis and LTBI.

- Not effected by BCG.

Implementing policy recommendations



THE
STOP TB
DEPARTMENT



Patients with TB products

Sputum

Urine

Pus

Body fluid

Direct Exam

PCR + Drug Resistance

Culture

Patients suspected TB but No TB products

- TST
- QuantiFERON TB gold Test
- T-Spot Test.

TB distribution in Syria 1/1-30/6/2010

المحافظات	خارج الرئة	رئوي	المجموع الكلي
حلب	320	426	746
دمشق	145	60	205
درعا	30	21	51
دير الزور	67	65	132
حمّاة	38	45	83
الحسكة	83	60	143
حمص	69	56	125
ادلب	50	43	93
اللاذقية	53	58	111
القنيطرة	9	5	14
الرقّة	60	65	125
ريف دمشق	196	82	278
السويداء	7	5	12
طرطوس	12	30	42
المجموع	1139	1021	2160



Thank you for your attention