

# Tuberculosis Infection – New Technology for an Old Disease

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# Tuberculosis (TB)

- 1500 BC TB bones/remnants of old TB adhesions in chest of Egyptian mummies
- 1546 AD Girolamo Tracastoro – first wrote that TB was contagious
- 1882 AD Robert Koch, a German bacteriologist, discovered TB
- 1924 AD Development of Bacillus Calmette-Guerin (BCG) vaccine
- 1945 AD Effective antimicrobial chemotherapy started (streptomycin)

# Famous People Who Reportedly Died of TB

King Tutankhamen

Cardinal Richelieu

Frederick Chopin

John Keats

Robert Louis Stevenson

Charlotte Bronte

Eleanor Roosevelt

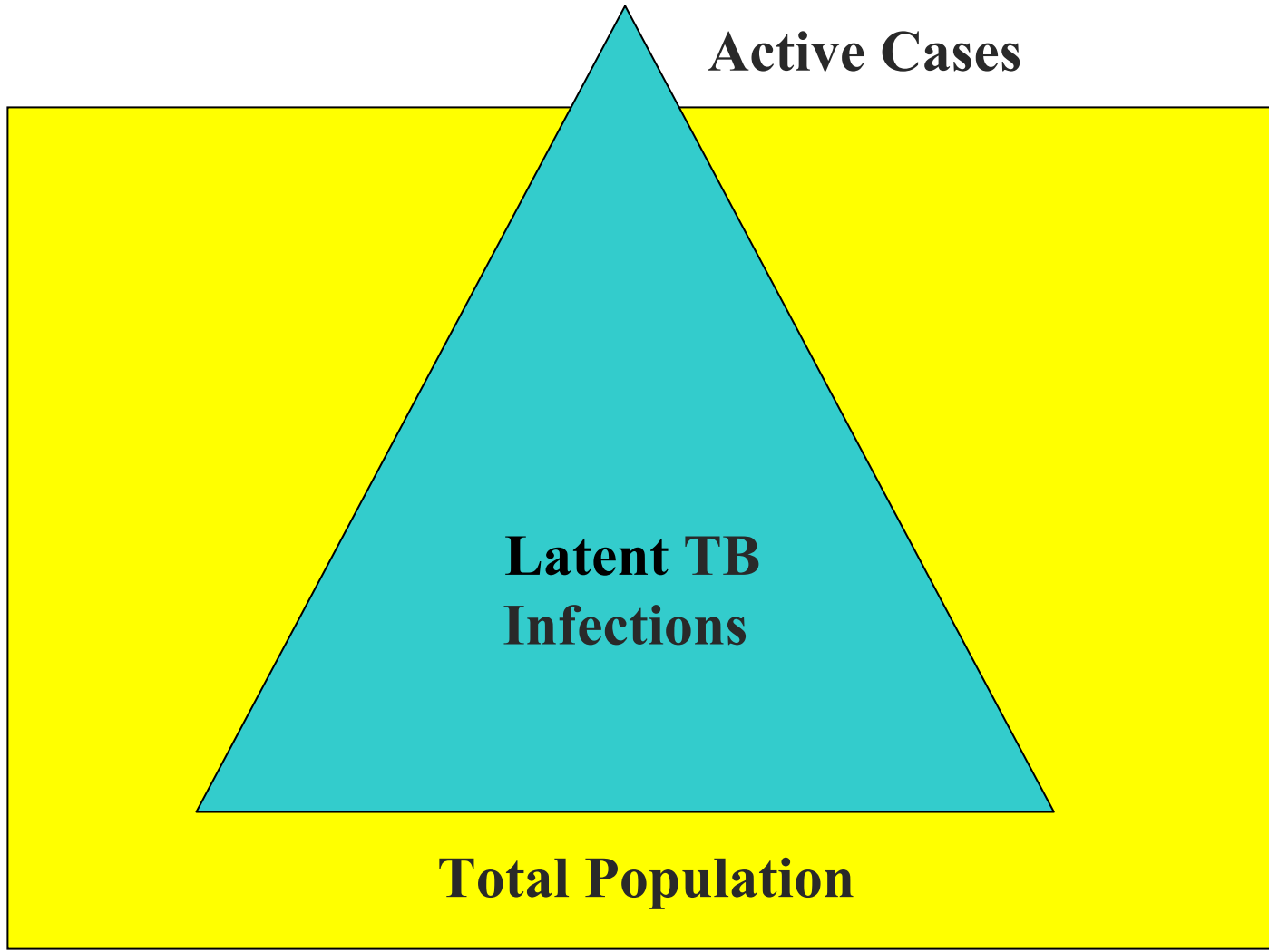
Edgar Allan Poe

George Orwell

Vivien Leigh

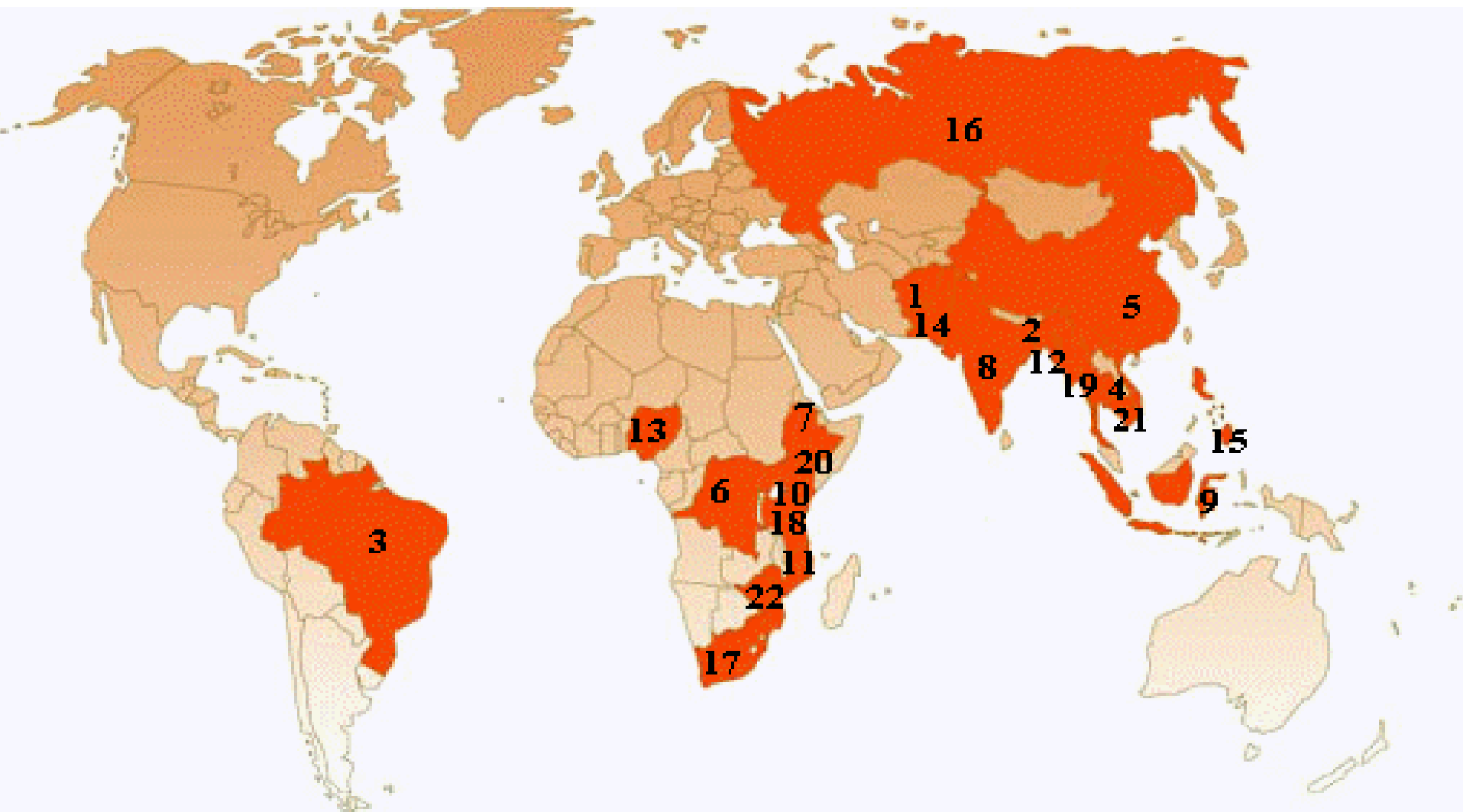
D.H. Lawrence

# Tuberculosis (Tip of the Iceberg)



# Tuberculosis Worldwide

- 2 billion latently infected people, worldwide
- 9 million new cases each year
- 2 million die each year (vs 200,000 from HIV)



# Tuberculosis in the U.S.

- 10 to 15 million people infected with latent TB
- 12,898 new cases of active TB in 2008
- Targeted screening and treatment
  - 18-20 million skin tests/year
  - 50% performed in hospitals



# Complexity Caused by Immigration

- TB rate 10 times greater among foreign-born
  - Foreign-born: 20.2 cases/100,000
  - U.S.-born: 2.0 cases/100,000
- 58.8% of all TB cases occurred in foreign-born individuals
- Higher number of foreign-born individuals are BCG vaccinated



MMWR. 2009;58:249.

# DRUG-RESISTANT TB

- MDR-TB—resistant to at least INH & Rifampin (500,000 new cases/year; 125 in U.S.—2008)
- XDR-TB—MDR plus resistance to all fluoroquinolones (cipro, etc.) + resistance to at least one of the 2<sup>nd</sup> line injectable drugs:  
Amikacin, Capreomycin, or Kanamycin (4 in U.S.—2008)

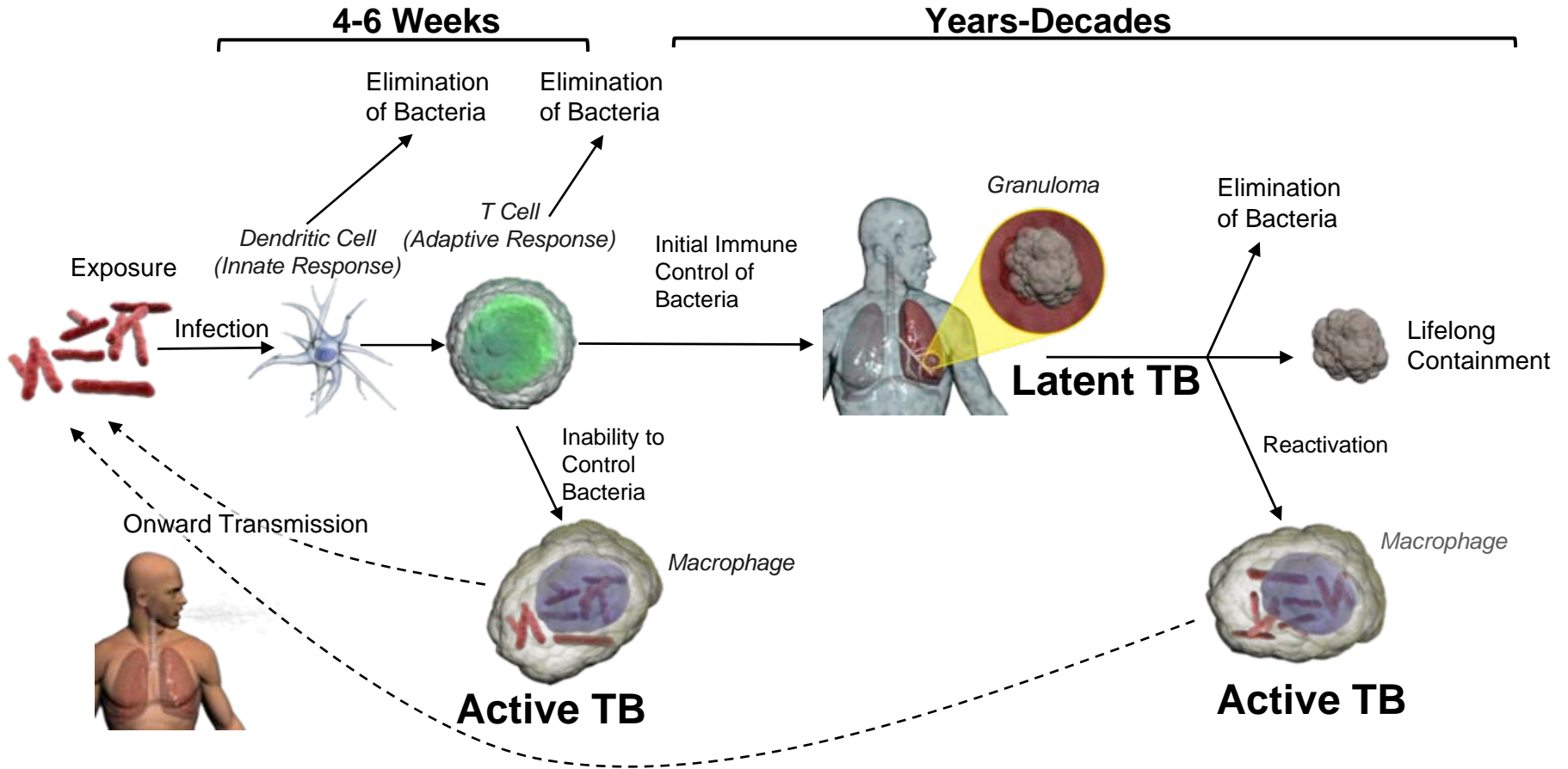
# Drug-resistant TB

- TDR-TB (Super XDR-TB)
  - Resistant to all 2nd line class drugs
- Iranian Study (*Chest.* 2009;136:420)
  - 146 MDR-TB
  - 8-XDR-TB
  - 15-TDR-TB
- In all TDR-TB cases, culture & smear remained (+) after 18 months of therapy with 2nd line drugs

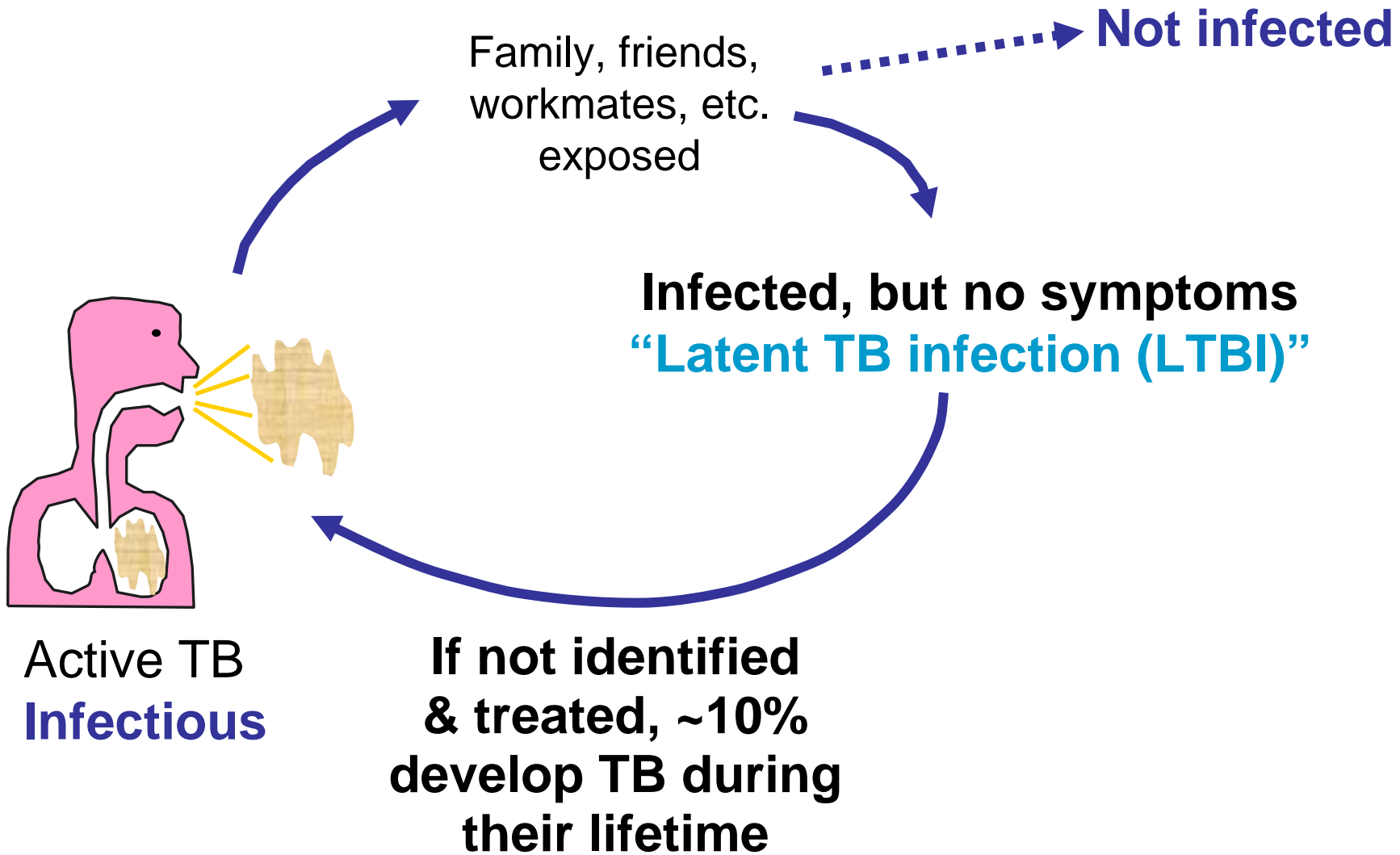
# Case Report

62 YO male hospital phlebotomist who underwent a routine employee QuantiFERON® screening test. The QuantiFERON® test was positive and patient was worked up by Employee Health Nurse for TB. Chest x-ray showed infiltrate in right lung. Bronchoscopy was performed and samples collected for AFB studies. AFB smear was negative; however, culture was (+) for TB. Phlebotomist was removed from hospital duties and monitored by local County Health Department. Anti-TB medication was started and phlebotomist recovered uneventfully and returned to work. Subsequent County Health Department investigation revealed no TB transmission to other people/patients.

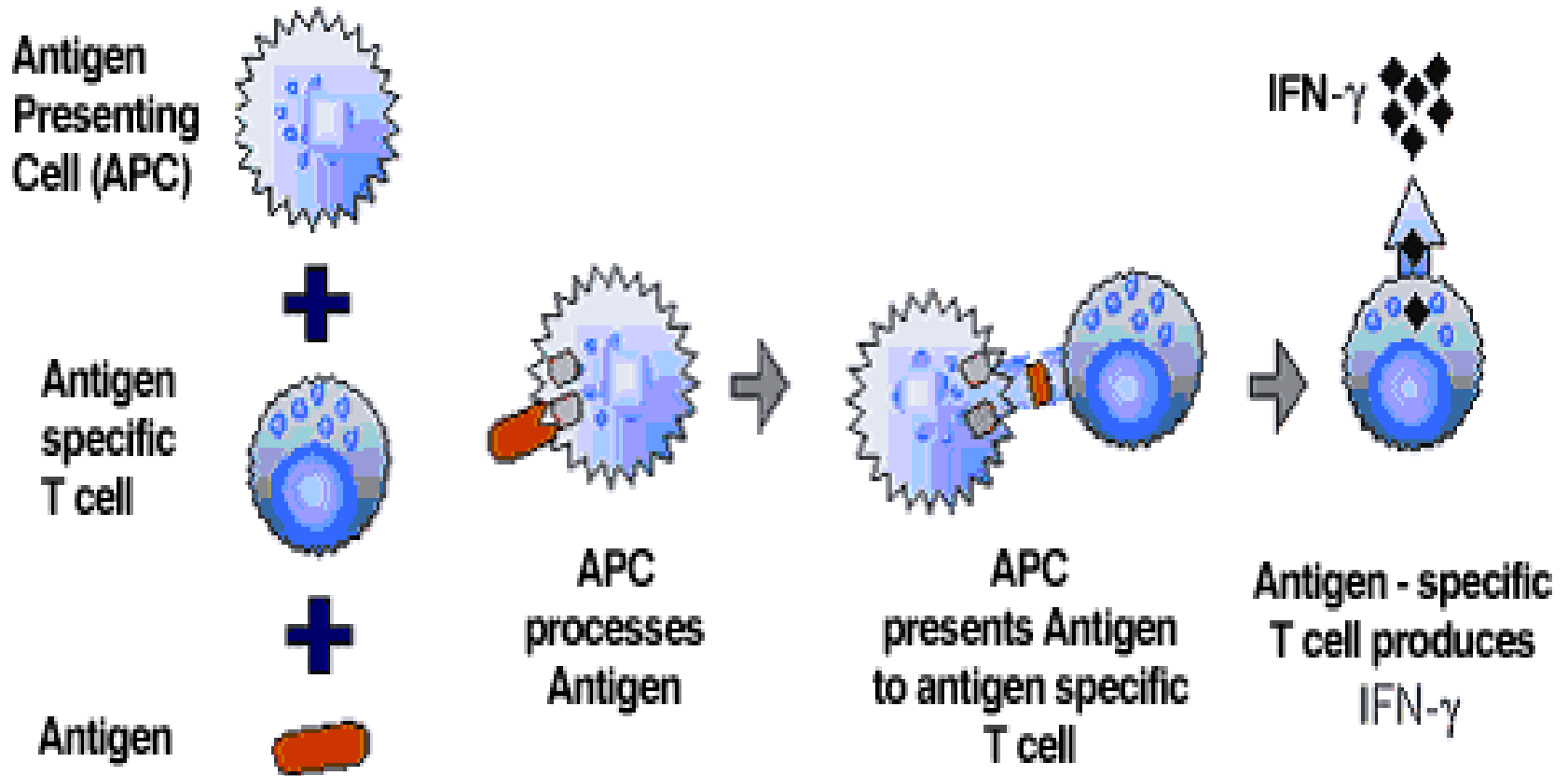
# Natural History of TB Infection



# Transmission of TB



# Interferon Gamma Release



# Active vs Latent TB

## Latent TB Infection

MTB present

Tuberculin skin test/IGRA +

Normal chest x-ray

Negative sputum smear,  
culture

No symptoms

Not infectious

Not defined as TB case

## Active TB in Lungs

MTB present

Tuberculin skin test/IGRA +

Lesion in chest x-ray (usually)

Positive sputum smear,  
culture

Cough, fever, weight loss

Often infectious before  
treatment

Defined as TB case



# TB Surveillance

High Risk of Transmission	High Risk of Progression/Reactivation (Immunosuppressed)	High Risk of Disease
<ul style="list-style-type: none"><li>■ Healthcare workers</li><li>■ Foreign-born</li><li>■ Prisoners</li><li>■ Chronic care residents</li><li>■ Military personnel</li><li>■ TB contacts</li></ul>	<ul style="list-style-type: none"><li>■ HIV</li><li>■ Rheumatoid arthritis</li><li>■ End-stage renal disease</li><li>■ Elderly</li><li>■ Children</li><li>■ Cancer chemotherapy</li><li>■ Organ transplant</li><li>■ Diabetes</li></ul>	<ul style="list-style-type: none"><li>■ TB suspects</li></ul>

# Diagnosis of Active TB Infections (Chantilly/SJC)

- AFB smears
- AFB culture
  - ID using sequencing/probes
- Amplification
  - TMA or PCR method
- Quantiferon® TB Gold

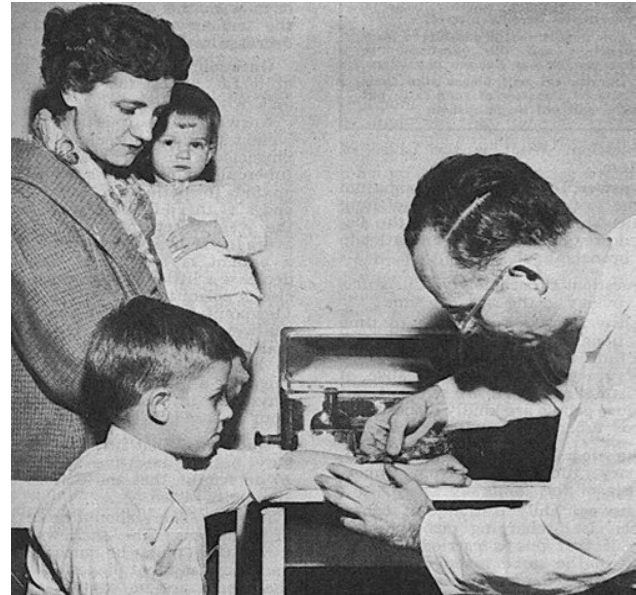
# Diagnosis of Latent TB Infections in U.S.

- TB skin test
- Interferon gamma release assays (IGRA)
  - QuantiFERON®
  - T-SPOT®.TB



# Tuberculin Skin Test (19<sup>TH</sup> Century Assay [1890])

- Purified protein derivative (PPD) is injected intradermally
- Measure size of reaction after 48-72 h
  - Induration (firm area)
  - Erythema (redness)



# Tuberculin Skin Test

## Limitations

- Subjectivity
- Moderate sensitivity
- Boosting
- Need for 2 visits
- Poor specificity
  - BCG vaccination
  - Non-TB mycobacteria (NTM)
  - Latency vs active infection



# Advantages of IGRA

- Provides high specificity:
  - antigens used in RD-1 & RD11 genes not found in the BCG vaccine
  - BCG vaccinated patients do not test positive
- More sensitive in immunocompromised patients than TB skin test
- Only 1 clinic visit required
- No “booster effect”

# TB Peptide Antigens

## ESAT-6, TB7.7, and CFP-10

- Encoded by RD-1 & RD11 genes
- Absent from BCG (TB-specific)
- Absent from most non-TB Mycobacteria
- Induce IFN- $\gamma$  responses
- TB7.7 (QuantiFERON<sup>®</sup> only)

# No Cross-Reactivity to BCG and Most NTMs

TB Complex	Antigens		Environmental Strains	Antigens	
	ESAT-6	CFP 10		ESAT-6	CFP 10
<i>M. tuberculosis</i>	+	+	<i>M. abcessus</i>	-	-
<i>M. africanum</i>	+	+	<i>M. avium</i>	-	-
<i>M. bovis</i>	+	+	<i>M. branderi</i>	-	-
BCG substrain			<i>M. celatum</i>	-	-
<i>gothenburg</i>	-	-	<i>M. chelonae</i>	-	-
<i>moreau</i>	-	-	<i>M. fortuitum</i>	-	-
<i>tice</i>	-	-	<i>M. gordonae</i>	+ (-)	+ (-)
<i>tokyo</i>	-	-	<i>M. intracellulare</i>	-	-
<i>danish</i>	-	-	<i>M. kansasii</i>	+	+
<i>glaxo</i>	-	-	<i>M. malmoense</i>	-	-
<i>montreal</i>	-	-	<i>M. marinum</i>	+	+
<i>pasteur</i>	-	-	<i>M. oenavense</i>	-	-
			<i>M. scrofulaceum</i>	-	-
			<i>M. smegmatis</i>	-	-
			<i>M. szulgai</i>	+	+
			<i>M. terrae</i>	-	-
			<i>M. vaccae</i>	-	-
			<i>M. xenopii</i>	-	-

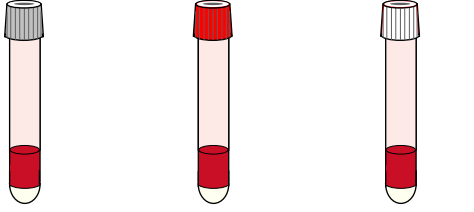
# Generations of QFT Testing

- **1<sup>st</sup> Gen:** QuantiFERON-TB (QFT)
  - FDA approved Nov. 2001
  - Test responds to PPD
- **2<sup>nd</sup> Gen:** QuantiFERON-TB Gold (QFT-G)
  - FDA approved May 2005
  - Tests responds to 2 TB proteins (ESAT-6, CFP-10)
- **3<sup>rd</sup> Gen:** QuantiFERON-TB Gold In-Tube (QFT-GIT)
  - FDA approved Oct. 2007
  - Test responds to 3 TB proteins (ESAT-6, CFP-10, TB7.7)



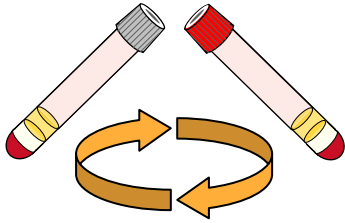
# QuantiFERON<sup>®</sup>-TB Gold In-Tube

## Stage 1 – Blood Incubation and Harvesting



Nil Control      ESAT-6 CFP-10 TB 7.7      Mitogen Control

1. Collect 1 ml of blood (X3).  
Incubate 36-38°C (16-24 h).

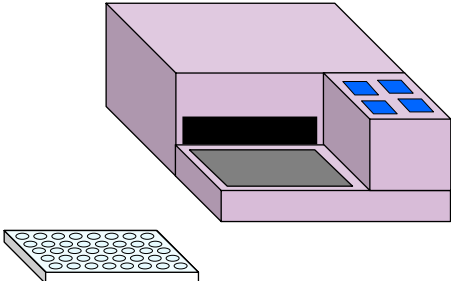
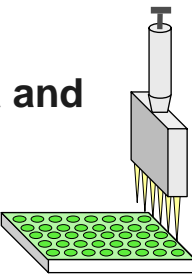


2. Centrifuge tubes for  
15 minutes.

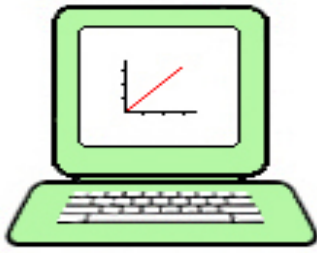
IFN- $\gamma$  stable  
refrigerated for at  
least 4 weeks.

## Stage 2 – Human IFN- $\gamma$ ELISA

3. Add plasma and conjugate to  
ELISA plate.  
Incubate for  
120 minutes  
at room temperature.



4. Wash and add substrate.  
Read absorbance after 30  
min.



5. Software calculates  
results and prints report.

The ELISA stage is easily automated on existing machines.

# QuantiFERON<sup>®</sup> Expected Results

Antigen Response	Result, IU/mL	Interpretation
Nil	0-0.5	Negative
Mitogen (PHA) – Nil	>0.5	Negative
TB – Nil	Not established	Negative
Nil	>0.8	Indeterminate
Mitogen (PHA) – Nil	<0.5	Indeterminate



# QuantiFERON<sup>®</sup> Expected Results

Antigen Response	Result, IU/mL	Interpretation
Nil	0-0.5 (normal)	Within limits
Nil	>0.8	Indeterminate
Mitogen (PHA) – Nil	>0.5	Negative
TB – Nil	Not established	Negative
Mitogen (PHA) – Nil	<0.5	Indeterminate



# QuantiFERON® Indeterminate Results

- **Incorrect handling of blood samples (probable lack of shaking/vortexing)**
- **Excessive levels of circulating IFN-gamma, presence of heterophile antibodies, or lymphocytes responding indiscriminately (poison ivy, rheumatoid arthritis, etc.) – redraw 1 month later & retest**
- **Low mitogen response (<0.1%); HIV CD4 counts <200 cells/mm<sup>3</sup>**
- **>16 h from blood draw to incubation in lab (36-38°C)**

# Gold Standard Test For Latent TB Infection?

***Does not exist***

***The IGRAs are measuring a lasting TB immune response & not specifically latent/active TB***

# TST & IGRA in TB Contact Investigations

## 2,004 Close Contacts with TB patients

- |  |   |
|--|---|
| <ul style="list-style-type: none"><li>■ 94% agreement between QFT and T-Spot<ul style="list-style-type: none"><li>■ QFT: 30%</li><li>■ T-Spot: 29%</li></ul></li></ul> | <ul style="list-style-type: none"><li>■ TST sensitivity<ul style="list-style-type: none"><li>■ &gt;10 mm: 72%</li><li>■ &gt;15 mm: 40%</li></ul></li><li>■ TST specificity: 65%</li></ul> |
|--|---|

**IGRAs are a more accurate indicator of LTBI than the TST. Using IGRAs rather than TST would decrease follow-up work by 70%.**

*Chest.* 2009;135:1010.

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11/30/2009

# TST & IGRA in Children

## 96 Children (Suspected/Risk of LTBI/Active)

- 93% agreement between QFT & T-SPOT
- Children (n=9) with clinical TB
  - QFT, 89%; T-SPOT, 100%; TST, 78%
- Children (n=38) with LTBI (+ TST) & (-) x-ray
  - QFT, 47%; T-SPOT, 39%

**High level of agreement between IGRA, but with the test results: (+) TST & (-) IGRA (challenging interpretation)**

# Predictive Value of QuantiFERON®

## 601 German contacts (exposed to TB)

- 11% QFT positive
- 40% TST positive

## Rate of progression to active TB disease

- 6/41 (14.6%) QFT + w/o prophylaxis progressed to active TB (1/6 was TST –)
- 5/219 (2.3%) TST + w/o prophylaxis progressed to active TB

**QFT is a better indicator of TB disease progression than TST testing.**

*Am J Respir Crit Care Med. 2008.*

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# TST & IGRAs in the Immunocompromised (Adults)

Assay	LTC (120)	HIV (116)	HM (95)
TST	20 (17%)	6 (5%)	10 (11%)
QFT	28 (23%)	5 (4%)	17 (18%)
TSPOT	32 (27%)	4 (4%)	25 (26%)

- T-SPOT (18%) and QFT (15%) more sensitive than TST (11%); no differences among HIV group
- Combined approach (TST plus IGRA) may be needed to maximize screening (i.e. HIV group)
- Higher # QFT indeterminate results vs T-SPOT; however, T-SPOT more technically complex

*Chest.* 2009;136:198.

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# Comparison of IGRA

Property	QFT	T-SPOT
FDA cleared	Yes	Yes
Draw to process time	<16 h	<8 h
Vendor-supplied transport tubes	Yes	No
Technically complex	No	Yes
Potential advantage in patients with low PBMC	No	Yes

# Disadvantages of IGRA

- Blood samples must be processed within 16 h (QFT) or within 8 h (TB-Spot) after draw; QFT 3 day stability if client incubated
- QFT needs vendor-supplied transport tubes
- T-Spot technically complex
- Don't differentiate between active/latent TB
- Booster effect: previous TB skin test?
- Limited data in
  - Children
  - Recent TB exposure
  - Immunocompromised (AIDS, etc.)

# Advantages of IGRAs

- Requires single patient visit
- Results available within 24 h
- Not subject to reader interpretation
- Not affected by prior BCG vaccination

# DETECTION OF LATENT TB INFECTIONS

- **TST only**
- **IGRA only**
- **TST plus IGRA (HIV, etc.)**

# Offered at Quest Diagnostics....

- Standard QuantiFERON<sup>®</sup> test
  - Transport tubes must be incubated within 16 h after draw
  
- Client-incubated QuantiFERON<sup>®</sup> test
  - Client must incubate transport tubes on-site [36-38°C] within 16 h after draw