Interferon Gamma Release Assays (IGRAs) in the Diagnosis of Tuberculosis

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ASCLS P.A.C.E.® Credit

- Laboratory professionals may earn 1.0 contact hour for participation in today's program
- Learning objectives: upon completion of this program, participants will be able to
  - Discuss tuberculosis statistics and trends in the US and worldwide
  - Identify the differences between active and latent tuberculosis and how each is diagnosed
  - Compare advantages/disadvantages of, and explain the latest CDC guidelines for, using IGRAs in the diagnosis of tuberculosis
  - Describe best practices in conducting and interpreting IGRAs

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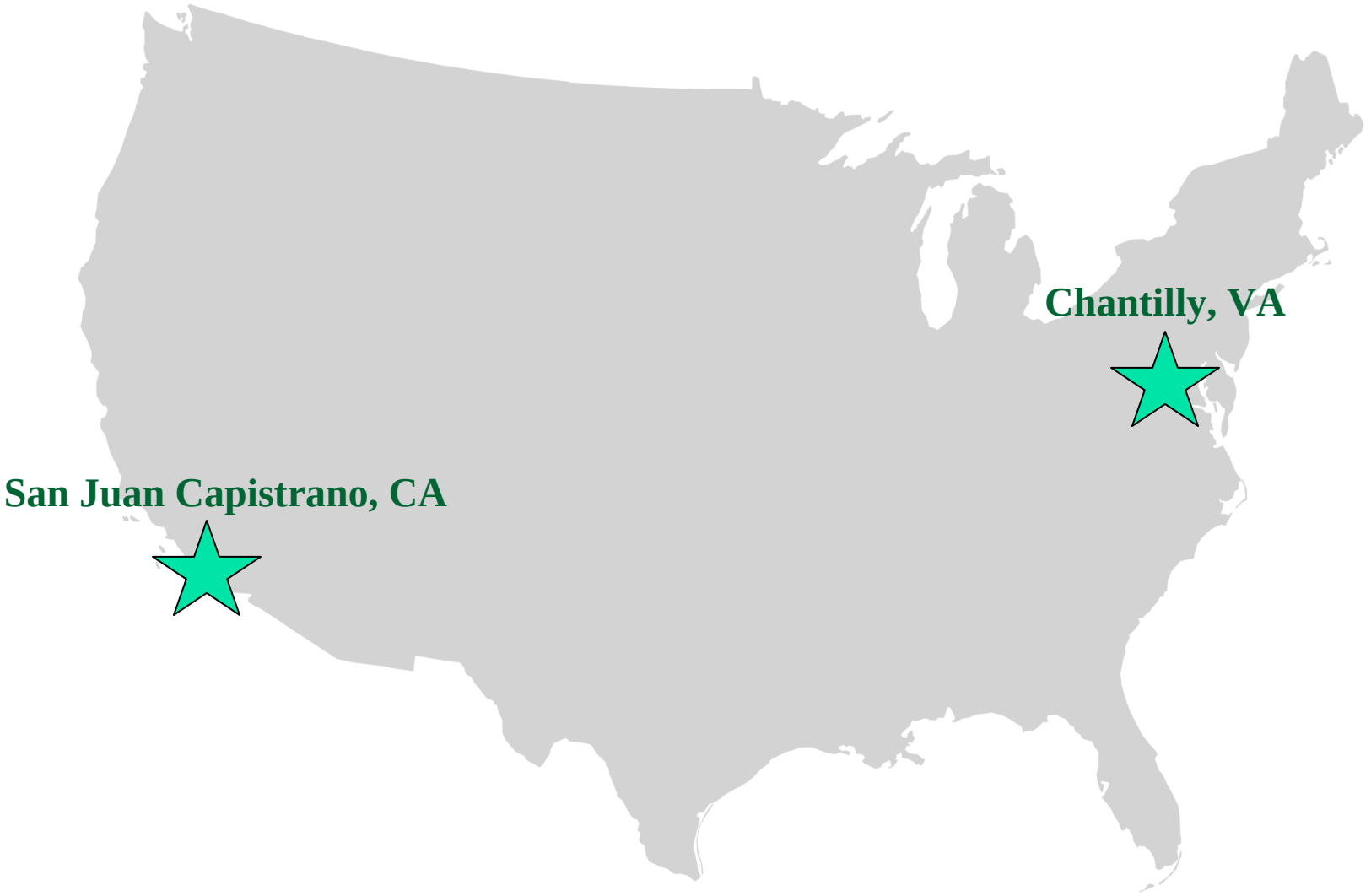

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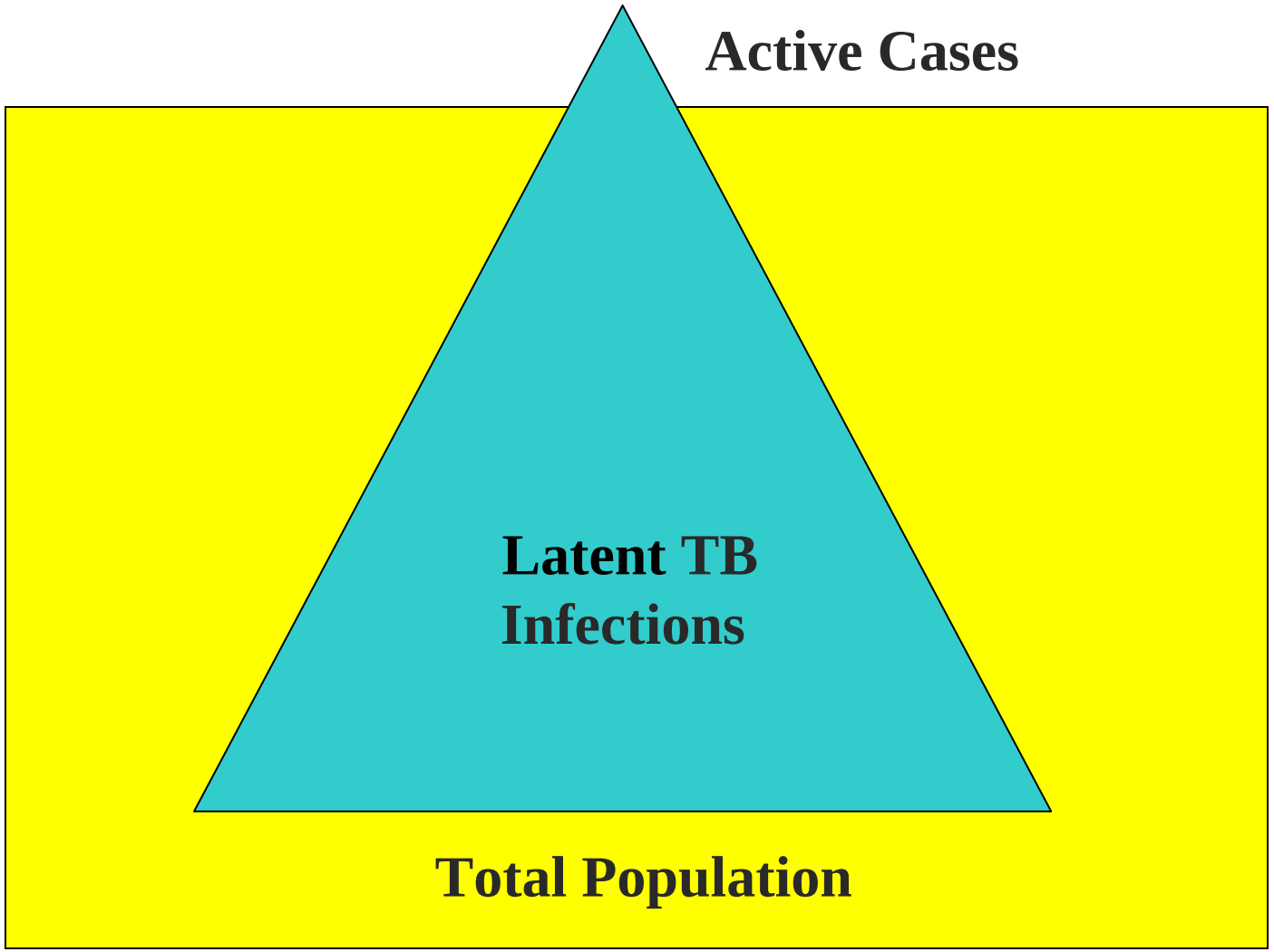
Tuberculosis (TB): Tip of the Iceberg



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Tuberculosis Worldwide

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

AAC 2009. 53, 849

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Tuberculosis in the US

- 10-15 million people infected with latent TB (4%)*
- 11,540 new cases of active TB in 2009 (11.4%) ↓
- Targeted screening and treatment
  - 18-20 million skin tests/year
  - 50% performed in hospitals

*AJRCCM, 2008, 177, 348

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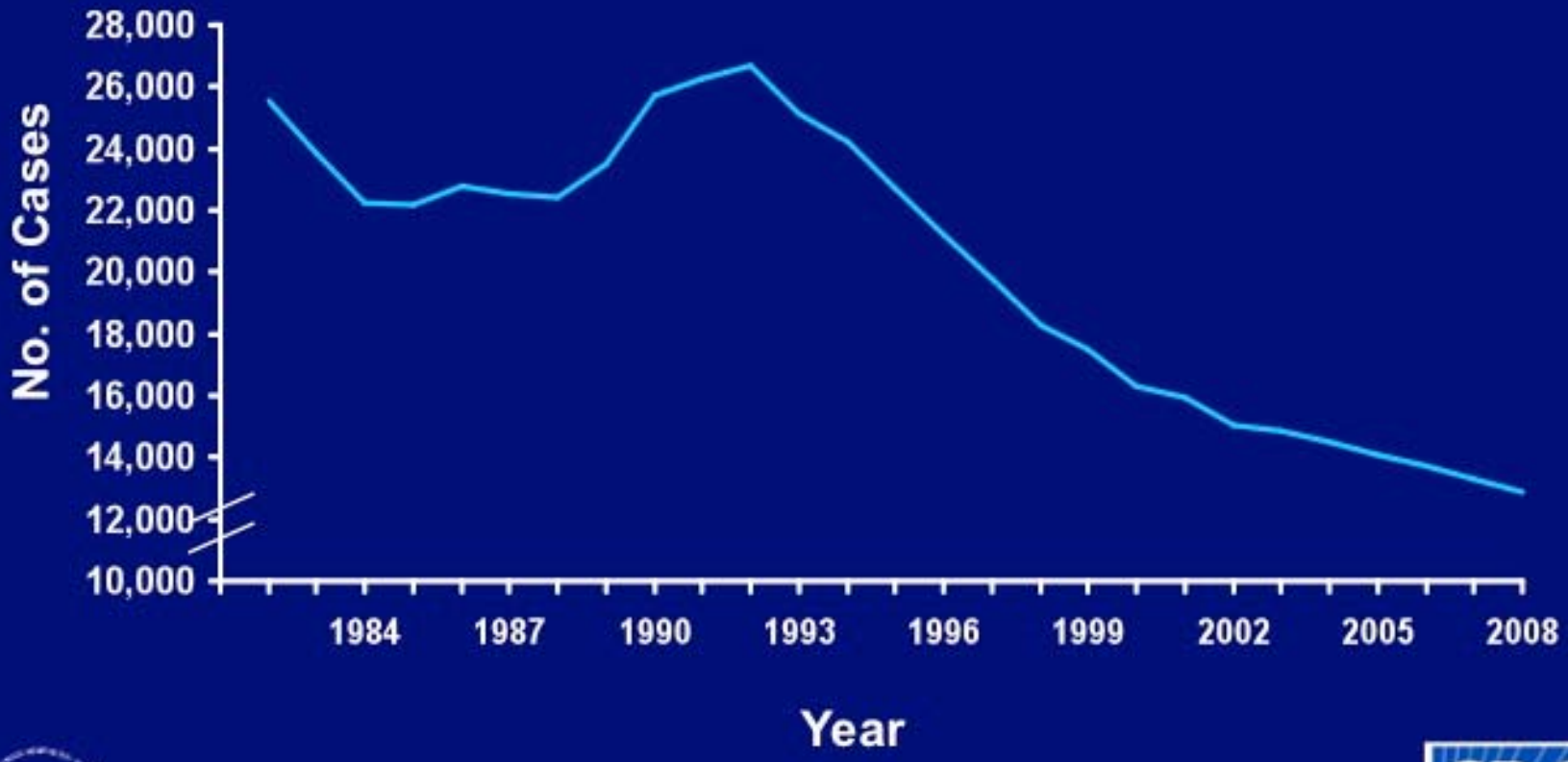
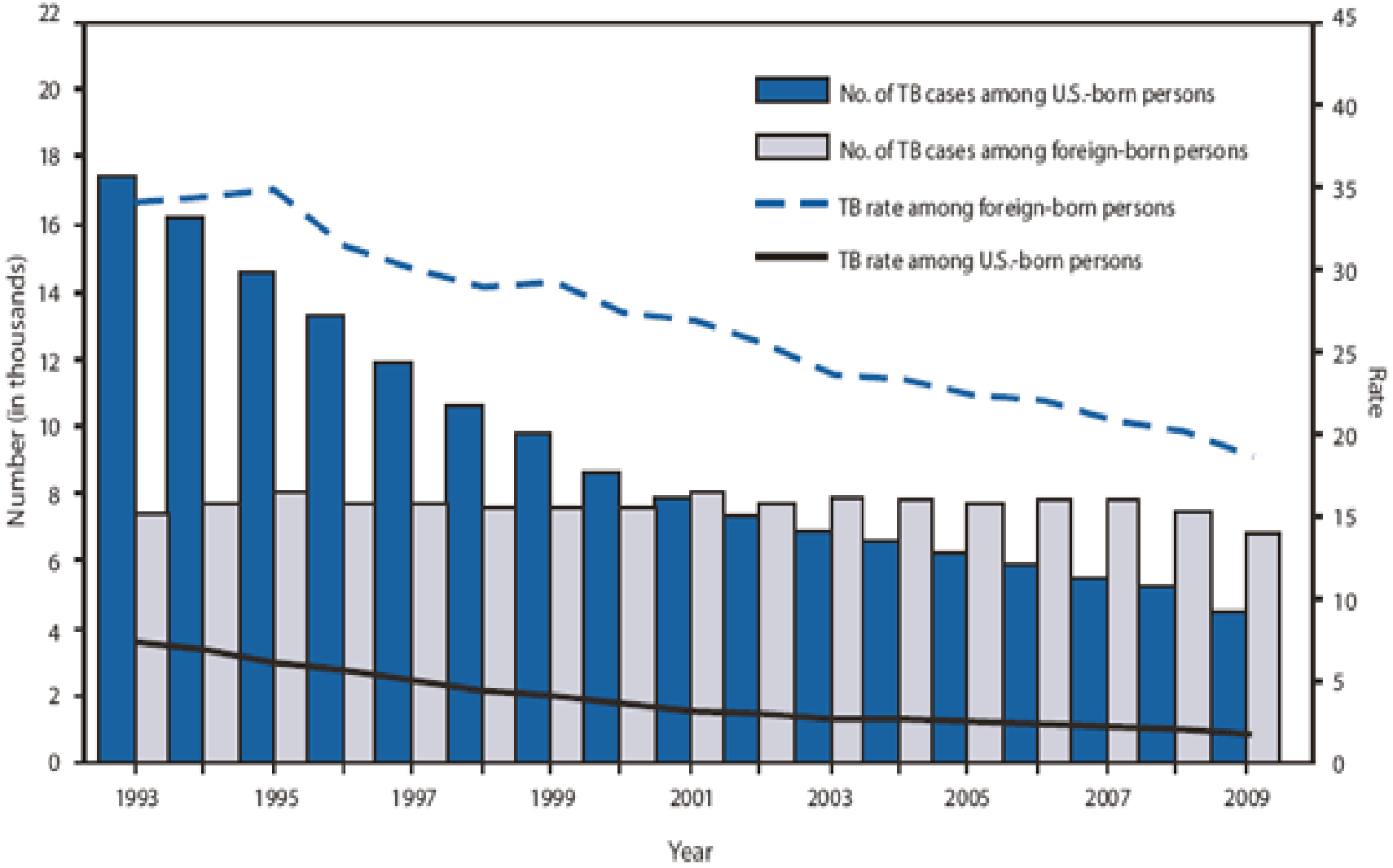
Reported TB Cases*
United States, 1982–2008



*Updated as of May 20, 2009.

Number and Rate* of TB Cases Among US-Born and Foreign-Born Persons by Year Reported: US, 1993-2009



MMWR, 2010, 59(10);289-294.

* Per 100,000 population

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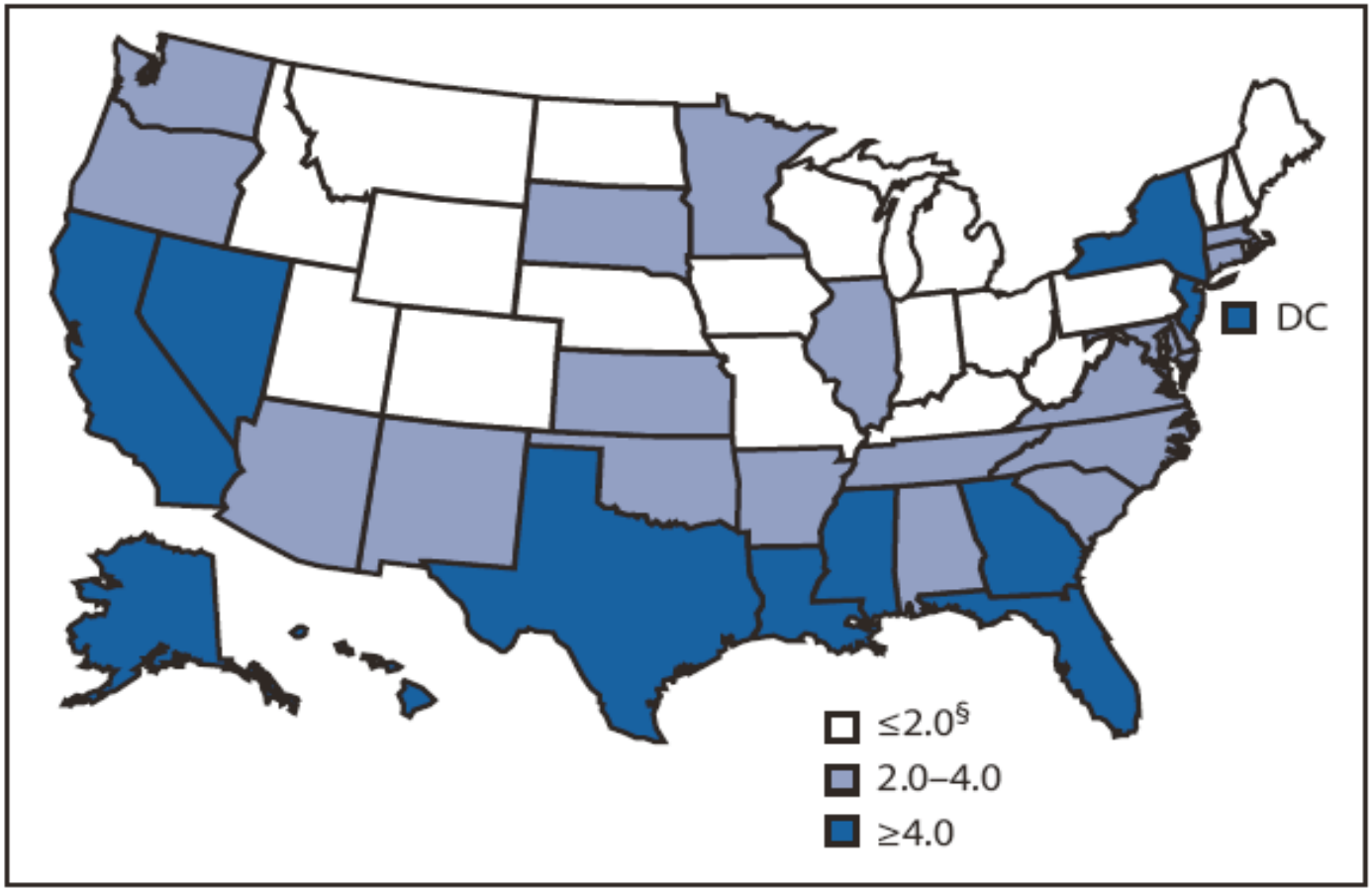
Rate* of TB cases by State/Area: US, 2009



MMWR, 2010, 59(10);289-294
SOURCE: National TB Surveillance System.
* Per 100,000 population

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Complexity Caused by Immigration

- TB rate 11 times greater among foreign-born
  - Foreign-born: 18.6 cases/100,000
  - US-born: 1.7 cases/100,000
- 58.9% of all TB cases occurred in foreign-born individuals
- Higher number of foreign-born individuals are BCG-vaccinated

MMWR, 2010, 59(10);289-294

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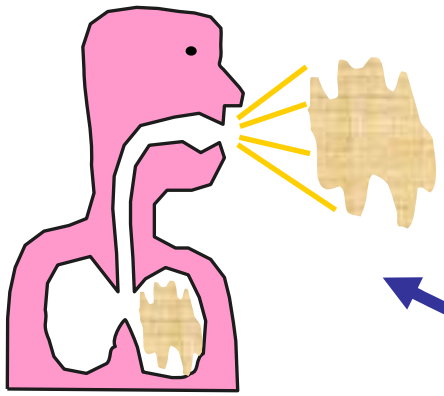
Transmission of TB

Family, friends, workmates, etc. exposed

Not infected

Infected, but no symptoms
"Latent TB infection (LTBI)"



Active TB
Infectious

If not identified & treated, ~10% develop TB during their lifetime

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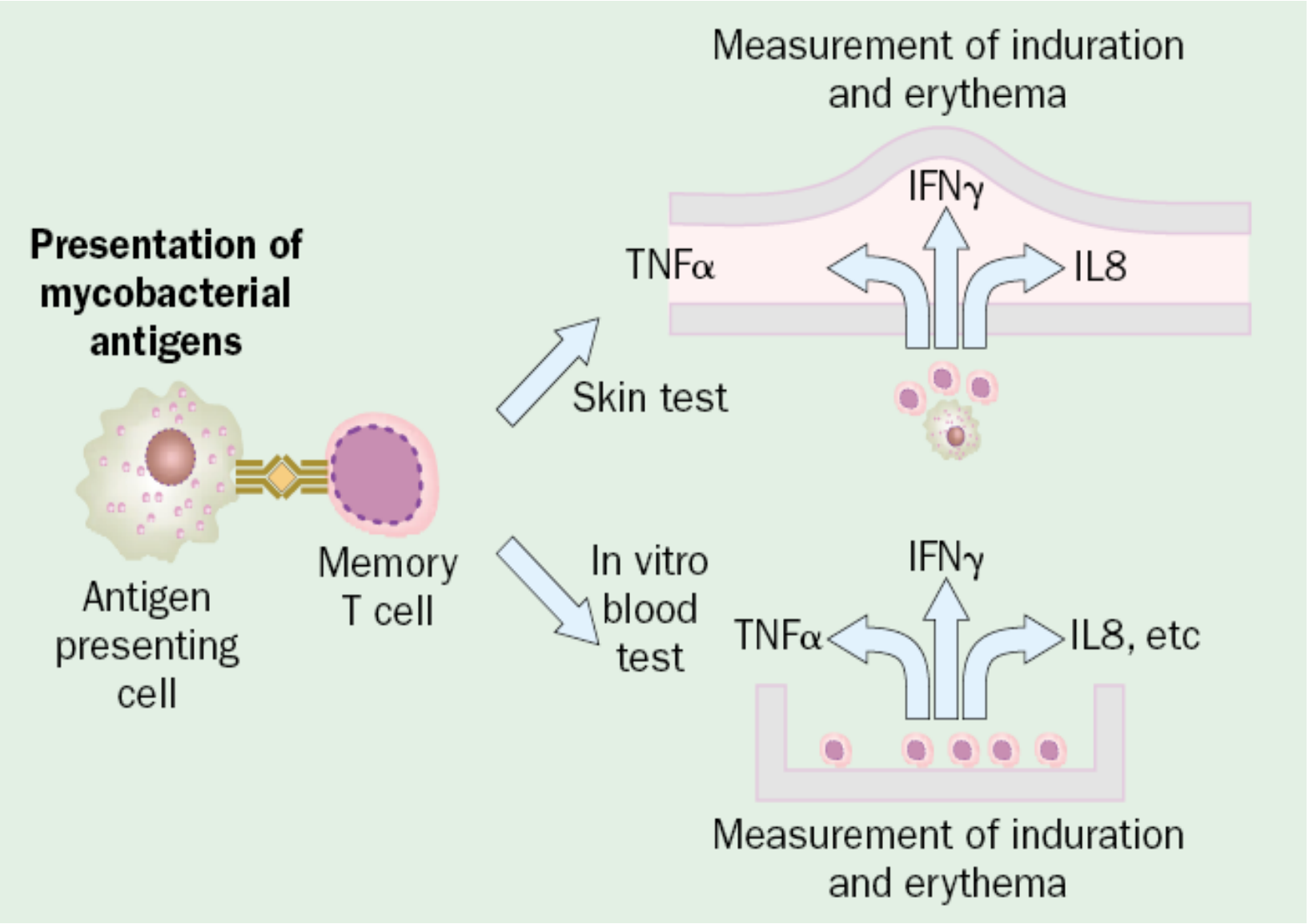

Image courtesy of Neil W. Schluger, M.D, Columbia University

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Active vs Latent TB

Latent TB Infection	Active TB in Lungs
MTB present	MTB present
Tuberculin skin test/IGRA +	Tuberculin skin test/IGRA +
Normal chest x-ray	Lesion in chest x-ray (usually)
Negative sputum smear, culture	Positive sputum smear, culture
No symptoms	Cough, fever, weight loss
Not infectious	Often infectious before treatment
Not defined as TB case	Defined as TB case

TB Surveillance

High Risk of Transmission	High Risk of Progression/Reactivation (Immunosuppressed)	High Risk of Disease
■ Healthcare workers	■ HIV	■ TB suspects
■ Foreign-born	■ Rheumatoid arthritis	
■ Prisoners	■ End-stage renal disease	
■ Chronic care residents	■ Elderly	
■ Military personnel	■ Children	
■ TB contacts	■ Cancer chemotherapy	
	■ Organ transplant	
	■ Diabetes	

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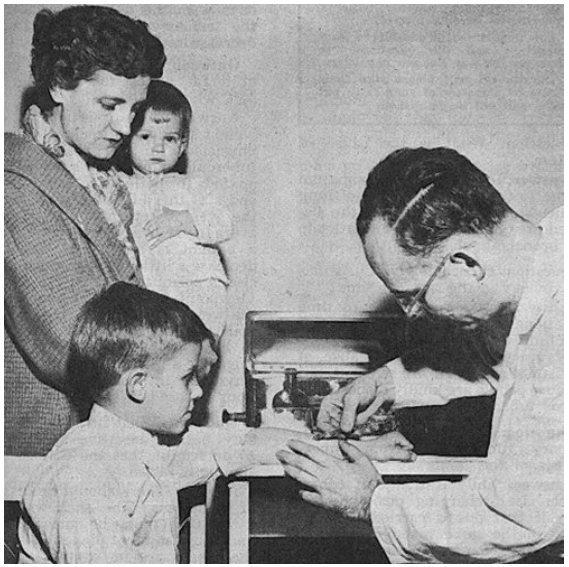
Tuberculin Skin Test
(In Routine Use Since 1910)

- Purified protein derivative (PPD) is injected intradermally (polyvalent mixture of >200 TB proteins)
- Measure size of reaction after 48-72 h
  - Induration (firm area)
  - Not erythema (redness)



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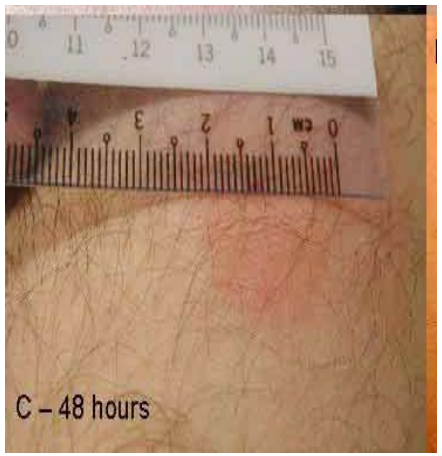
Tuberculin Skin Test

Limitations

- Reader variability (requires trained staff)
- Moderate sensitivity (esp. HIV, other immunocompromised patients)
- Variations of results (different anatomical site)
- Boosting
- Need for 2-4 visits
- Poor specificity
  - BCG vaccination (up to 80%)
  - Non-TB environmental mycobacteria (0.1-2%)
  - Latency vs active infection



Picture courtesy of Neil W. Schluger, M.D, Columbia University

What is the Cost of Evaluating and Treating People with False-Positive TSTs?

- Unnecessary return visits to the clinic
- Unnecessary chest radiographs
- Unnecessary blood tests
- Unnecessary INH hepatitis
- Unnecessary liver transplants
  - 2 liver transplants per year[1,2] in the U.S. from INH-induced acute hepatitis

[1]Russo et al. Liver Transplantation 2004; 10: 1018-1025
[2]MMWR 2010; 59: 224-229

Neil W. Schluger, M.D, Columbia University

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Diagnosis of Latent TB Infections in the US: A New ERA

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

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Centers for Disease Control and Prevention (CDC) 2010 Guidelines for Use of IGRA in Detection of LTBI

- Using IGRA for targeted testing:
  - As with the TST, IGRAs generally should not be used for testing persons who have a low risk for both infection and disease attributable to *M. tuberculosis* (except for those likely to be at increased risk in the future) because screening such persons diverts resources from activities of higher priority and increases the number of false-positive results.
  - If persons at low risk for both infection and progression are to be tested, selection of the test with the greatest specificity will minimize false-positive results, reduce unnecessary evaluation and treatment, and minimize the potential for adverse events from unnecessary treatment.

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Centers for Disease Control and Prevention (CDC) 2010 Guidelines for Use of IGRA in Detection of LTBI

- An IGRA is preferred for testing persons from groups that historically have poor rates of return for TST reading
- An IGRA is preferred for testing persons who have received BCG
- TST is preferred for testing children aged <5 years
- An IGRA may be used in place of TST (without preference) to test recent contacts of persons with infectious TB
- An IGRA or TST may be used for periodic screening that addresses occupational exposure to TB (eg, surveillance programs for health-care workers) with special considerations regarding conversions and reversions

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Dial-in: (888) 283-3954; Passcode: 9092010

Advantages of IGRAs

- Provides high specificity:
  - Antigens (RD-1 & RD11 genes) used not found in the BCG vaccine
  - BCG vaccinated patients do not test positive
- Only 1 clinic visit required
- No “booster effect”

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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-γ responses
- TB7.7 (QuantiFERON® only)

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No Cross-Reactivity to BCG and Most NTMs Among IGRAs

Tuberculosis Complex	ESAT-6	CFP-10	TB7.7*
M tuberculosis	+	+	+
M africanum	+	+	+
M bovis	+	+	+
BCG substrain			
gothenburg	-	-	-
moreau	-	-	-
tice	-	-	-
tokyo	-	-	-
danish	-	-	-
glaxo	-	-	-
montreal	-	-	-
pasteur	-	-	-

Environmental strains	ESAT-6	CFP-10	TB7.7*
M abcessus	-	-	-
M avium	-	-	-
M branderi	-	-	-
M celatum	-	-	-
M chelonae	-	-	-
M fortuitum	-	-	-
M gordonii	-	-	-
M intracellulare	-	-	-
M kansasii	+	+	-
M malmoense	-	-	-
M marinum	+	+	-
M oenavense	-	-	-
M scrofulaceum	-	-	-
M smegmatis	-	-	-
M szulgai	+	+	-
M terrae	-	-	-
M vaccae	-	-	-
M xenopi	-	-	-

*QuantiFERON only

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QuantiFERON In Tube (QFT-IT)

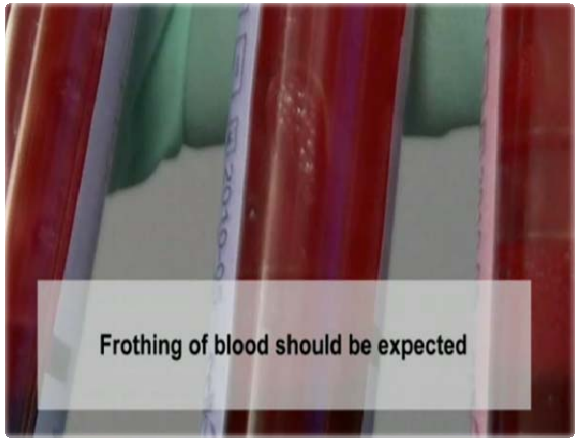
In the field:

- TB-specific antigen, Nil & mitogen tubes
- Collect blood directly into tubes

Field/Lab: Incubate and centrifuge

In the lab:

- ELISA for detection of IFN-gamma



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QFT-IT

- Sampling shipping/logistics
  - ***Collect blood, then ship to lab:*** Sample must be received with 16 hours of blood collection or…
  - ***Collect blood, incubate, then ship to lab*:** Incubated blood samples are stable for up to 3 days at RT/refrigerated temperatures (2-27°C) or…
  - ***Collect blood, incubate, centrifuge, then ship to lab*:** Sample is stable for up to 28 days at refrigerated temperatures (2-8°C)

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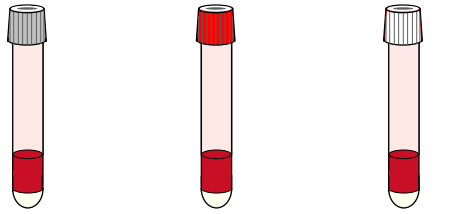
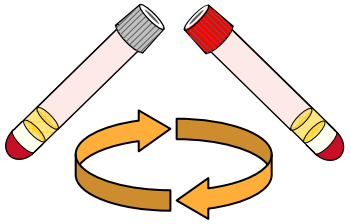
QuantiFERON-TB Gold In-Tube

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 36-38ºC (16-24 h).

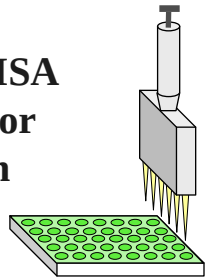


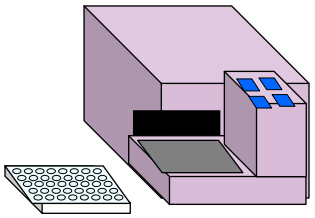
2. Centrifuge tubes at 2,000-3,000 x g for 5 minutes.

IFN-γ stable refrigerated for at least 4 weeks.

Stage 2 – Human IFN-γ ELISA

3. Add plasma and conjugate to ELISA plate. Incubate for 120 min. 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.

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QuantiFERON Results at Quest Diagnostics

- Reports contain a qualitative result and if desired, 3 quantitative results
- Possible qualitative results are:
  - Detected (positive)
  - Not Detected (negative)
  - Indeterminate
- Three quantitative results:
  - Nil (0-0.5)
  - Mitogen- Nil (≥0.5)
  - TB Antigen – Nil **(≥0.35 IU/ml = Positive)**

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QuantiFERON Indeterminate Results
(Low Mitogen Tube Response)

Technical Factors

- Incorrect handling of blood samples (probable lack of shaking) – PHA not adequately solubilized
- Not incubating samples
- >16 h from blood draw to incubation in lab (36-38ºC); sometimes as little as 6 hr can be detrimental to test
- Storage of filled blood collection tubes outside recommended range (22ºC ± 5ºC)

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QuantiFERON Indeterminate Results
(High Nil Tube Response)

Host Factors

- Presence of heterophile antibodies (human anti-mouse)
- Intrinsic gamma interferon secretion
- Recent vaccination(s)
- Lymphocytes responding indiscriminately (poison ivy, rheumatoid arthritis, etc.) – recommendation is to redraw one month later

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Centers for Disease Control and Prevention (CDC) 2010 Guidelines for Use of IGRA in Detection of LTBI

- Both the standard qualitative test interpretation and the quantitative assay measurements should be reported, together with the criteria for test interpretation, which will permit more refined assessment of results and promote understanding of the tests

TEST PATIENT 99999999/0 34 YEARS MALE

COLLECTED: 02/08/2010 00:00 HOSPITAL X
RECEIVED: 02/08/2010 URGENT CARE CENTER
REPORTED: 02/10/2010 2000 MAIN ST
2010/ 0/ 14800/ 0/32026366 CHANTILLY VA 20152
PT PHONE #:

----------TESTS----------RESULTS-FLAG--------REF. RANGE------UNITS

19453/Chantilly
Quantiferon(R)-TB Gold ITM

Quantiferon	DETECTED *	(Not Detected)
Nil	0.43	IU/mL
Mitogen-Nil	>10.00	IU/mL
TB Ag-Nil	>10.00	IU/Ml

The Nil value adjusts for patient sample background, heterophile antibody effects, or non-specific IFN. The Mitogen serves as a patient positive control. The result "Not Detected" or "Detected" is calculated from these values using an FDA-approved algorithm run on Quantiferon software.

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T-SPOT.TB Method

BD Sodium Citrate
Vacutainer® CPT™

BD Vacutainer®
Lithium
Heparin PST™

Greiner Bio-One
Lithium Heparin
Vacuette®

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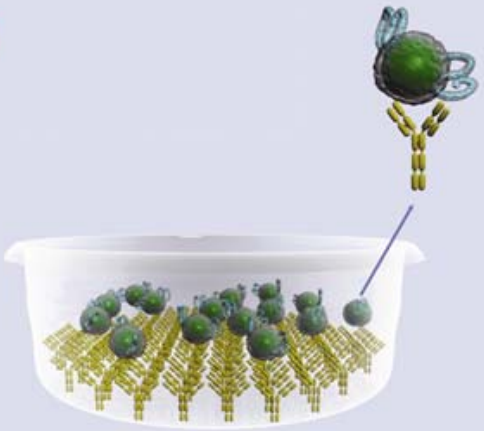
T-SPOT.TB Method

1.

Collect the blood sample. At the lab, PBMCs are separated from whole blood, washed, counted and inoculated into 4 separate microtiter wells.

2.



PBMCs [●] and specific TB antigens [ ] are added to wells pre-coated with antibodies to IFN-*y* [Y] and incubated 16 to 20 hours (37o C, CO2).

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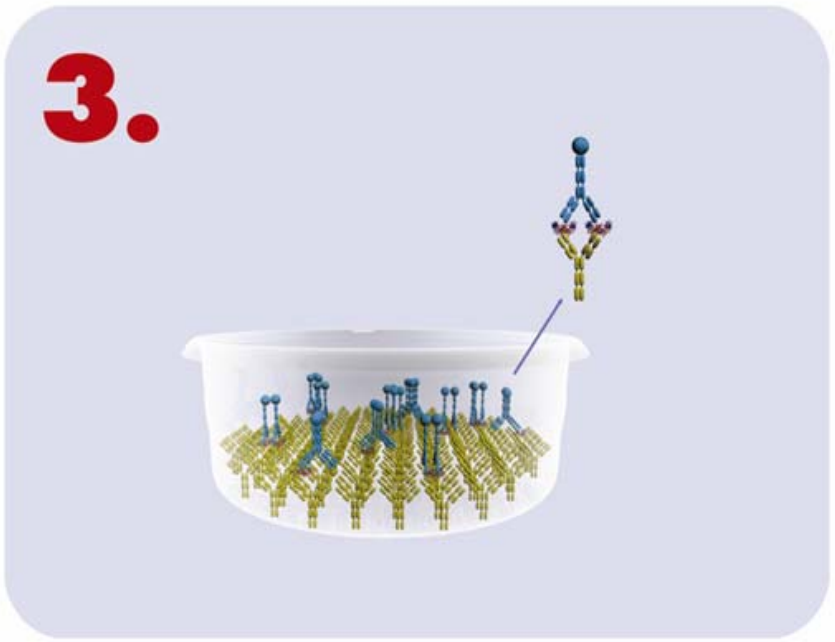
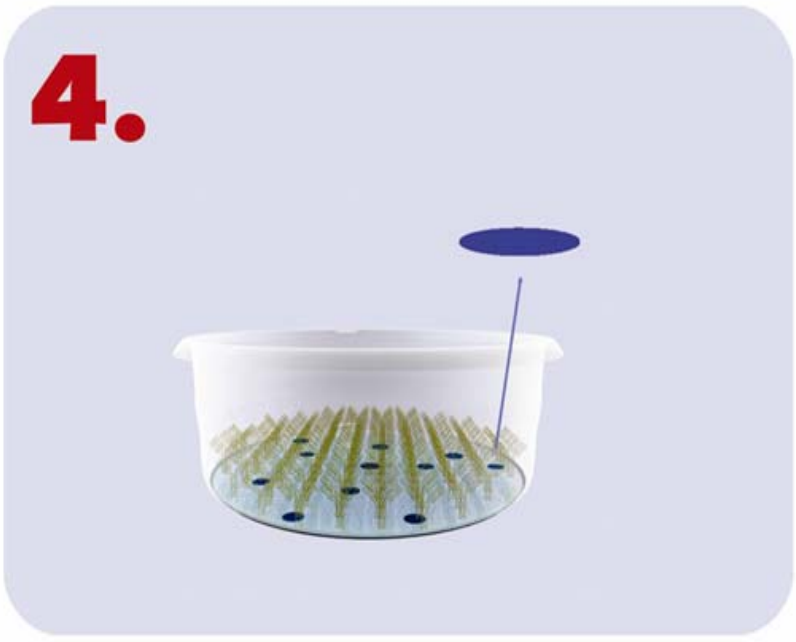
T-SPOT.TB Method



IFN-*y* [ ] is released from activated T cells and captured. Wash wells, add secondary conjugated antibody [ ]. Incubate for one hour.



Wells are washed. A substrate is added which produces spots [ ] where interferon gamma was secreted by T cells. Spots are counted.

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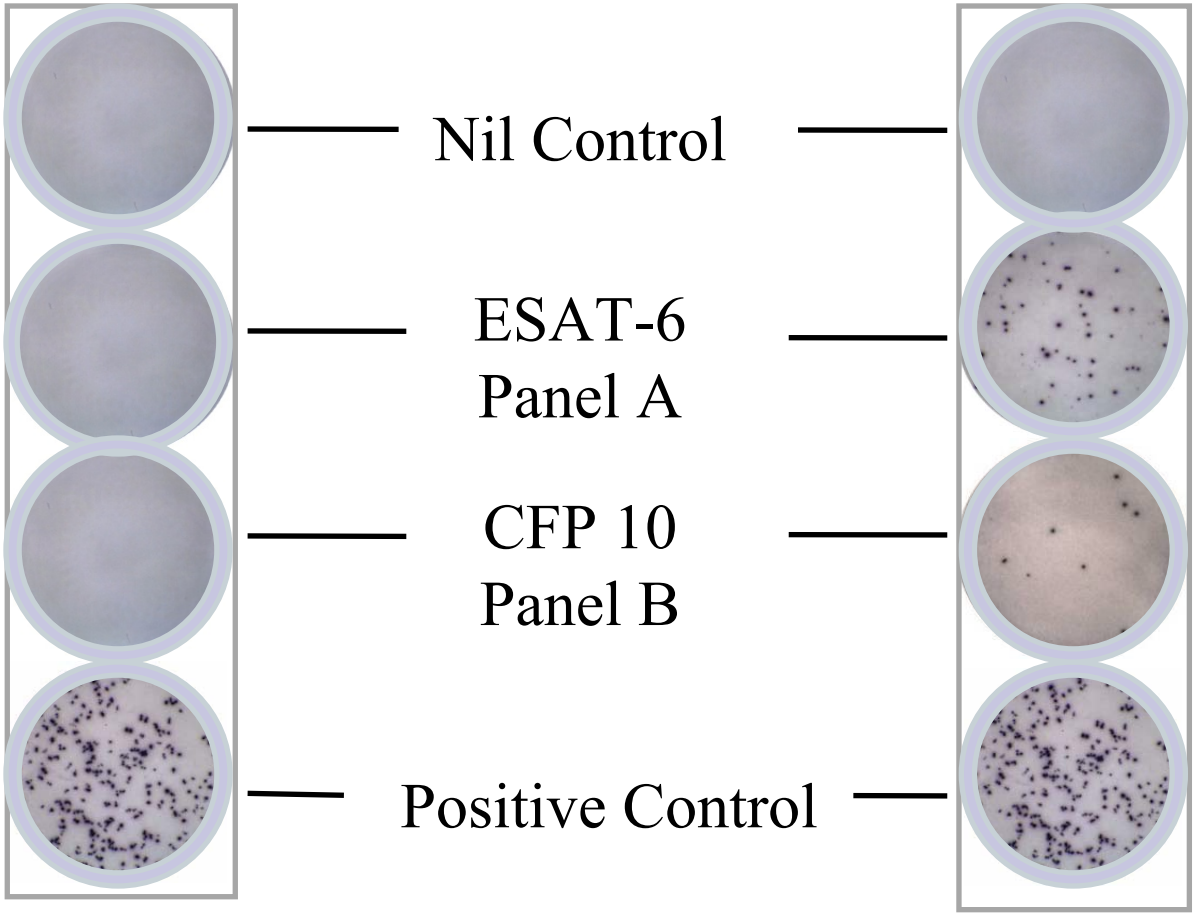
Interpretation of T-SPOT.TB Results



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Interpretation of T-SPOT.TB Results

- Positive: Panel A–NIL and/or Panel B–NIL ≥8 spots
- Borderline (equivocal) when higher of Panel A–NIL or Panel B–NIL spot count is 5, 6, or 7; retesting by collecting another sample is recommended
- Negative if Panel A–NIL and/or Panel B–NIL ≤4 spots; includes values <0

Video on T-Spot
http://www.youtube.com/watch?v=QBB2rcDGjbg&NR=1

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TST vs. QFT-IT vs. T-SPOT

Diagnosing Active TB compared to the Gold Standard: Positive Smear and/or Positive Culture

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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.

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Sensitivity of TST/IGRA Tests (Active TB Cases)

	Pooled Estimates		
Assay	**All Studies**	**No. Studies**	**Mix** Developed,Developing
TST	**70% (67-72%)**	**25**	**23, 2**
QFT-IT	**81% (78-83%)**	**19**	**13, 6**
QFT-IT	**84% (81-87%)**	**13**	**13, 0**
QFT-IT	**74% (69-79%)**	**6**	**0, 6**
T-SPOT	**87% (85-90%)**	**17**	**15, 2**

Diel, Loddenkemper, Nienhaus, Chest 2010;137;952-968

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Sensitivity of TST/IGRA Tests (Active TB Cases)

Study	Patient Population	QFT-IT	T-SPOT
1	Children	93%	93%
2	Adults & Children	79%	86%
3	Children	80%	56%
4	Adults	93%	62%
Total		84.3% (91 patients)	77.8% (84 patients)

Diel, Loddenkemper, Nienhaus, Chest 2010;137;952-968

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Specificity of TST/IGRA Tests (Population in Low Incidence Area With No Known Exposure to TB)

	Pooled Estimates	
Assay	**All Studies**	**No. of Studies**
TST	Not stated	
QFT	99% (98-100%)	5
T-SPOT	86% (81-90%)	3

Note: T-SPOT Cut-off of ≥6 spots used

FDA cleared Pkg insert is ≥8

Chest, 2010,137, 952

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QFT-IT & T-SPOT Indeterminate Results

Host Factors

- Compromised immune status of test individual (ie, HIV CD4 counts <200 cells/mm^3): QFT-IT
- Insufficient lymphocytes
- Inability of patient's lymphocytes to generate gamma interferon
- Extremes of age (<5 and >80 years old)
- Lack of response to PHA by some individuals (<0.1%): QFT-IT

Indeterminates: QFT-IT vs. T-SPOT

Patients	QFT-IT	T-SPOT
Pooled	2.14	3.8
Immunocompromised	4.42	6.12

Diel, Loddenkemper, Nienhaus, Chest 2010;137;952-968

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Gold Standard Test For Latent TB Infection?

Does not exist

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

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TST versus IGRA Tests in Contacts (LTBI)

TST Results mm	QFT % Pos.	T-SPOT % Pos	% Both Pos (No. discordant)
6-10	19.8	17.4	16.2
11-15	80.8	80.8	75.3
>15	97.4	97.4	**97.4 (0)**
6-10	9.8	9.5	6.3
11-15	38.8	34.7	32.7
>15	78.9	76.3	**76.3 (1)**

Overall agreement between both IGRAs = 93.9 %

Diel et al. Chest 2009; 135;1010-1018

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Specificity of TST/IGRA (LTBI)

	Pooled Estimates		
Assay	**All Studies**	**BCG-Vaccinated**	**Not Vaccinated**
TST	**66%(46-86%)**	**56% (34-78%)**	**98% (96-100%)**
QFT	**97% (95-99%)**	**96% (93-99%)**	**100% (94-100%)**
T-SPOT	**92% (88-95%)**	–	–

Ann Intern Med. 2007;146:340.

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Employee Services

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Cost Analysis of TST vs QFT

- 76 employees (new and hospital employees)
  - TST +/QFT +: 14
  - TST +/QFT –: 17
  - TST –/QFT +: 0
  - TST–/QFT–: 45
- Cost/test
  - TST: $22
  - QFT: $45
  - Chest x-ray: $180

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Cost Analysis of TST vs QFT

- Assume TST + gold standard:

TST cost (76 x $22)	$1,672
Chest x-ray cost (31 x $180)	$5,580
Total cost	$7,252

- Assume QFT + gold standard:

QFT cost (76 x $45)	$3,420
Chest x-ray cost (17 x $180)	$3,060
Total cost	$6,480

- Savings: $10.16 (per tested employee)

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DISCORDANT QFT RESULTS

HIV PATIENT

(What is the correct result?)

Results (IU/mL)				
Date	**Nil**	**Mitogen -Nil**	**TB Ag-Nil**	**Result**
4/25/09	**0.35**	**>10**	**0.09**	**Negative**
4/28/10	**0.25**	**>10**	**0.510**	**Positive**
5/17/10	**0.480**	**>10**	**0.310**	**Negative**

(+) = TB Ag-Nil ≥0.35 IU/mL)

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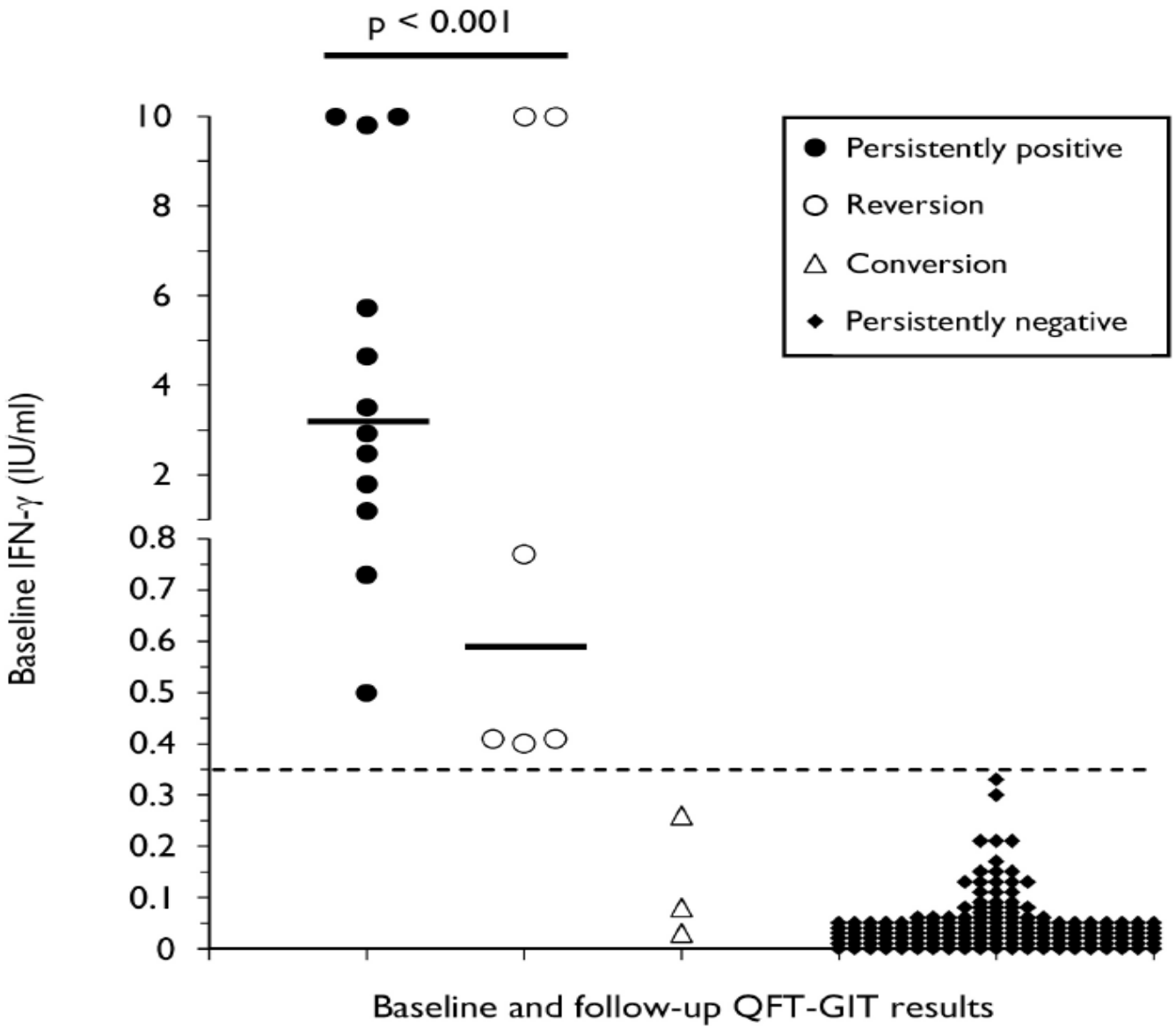
QFT Results in Serial Testing of HCWs



BMC Infectious Diseases 2010, **10**:220

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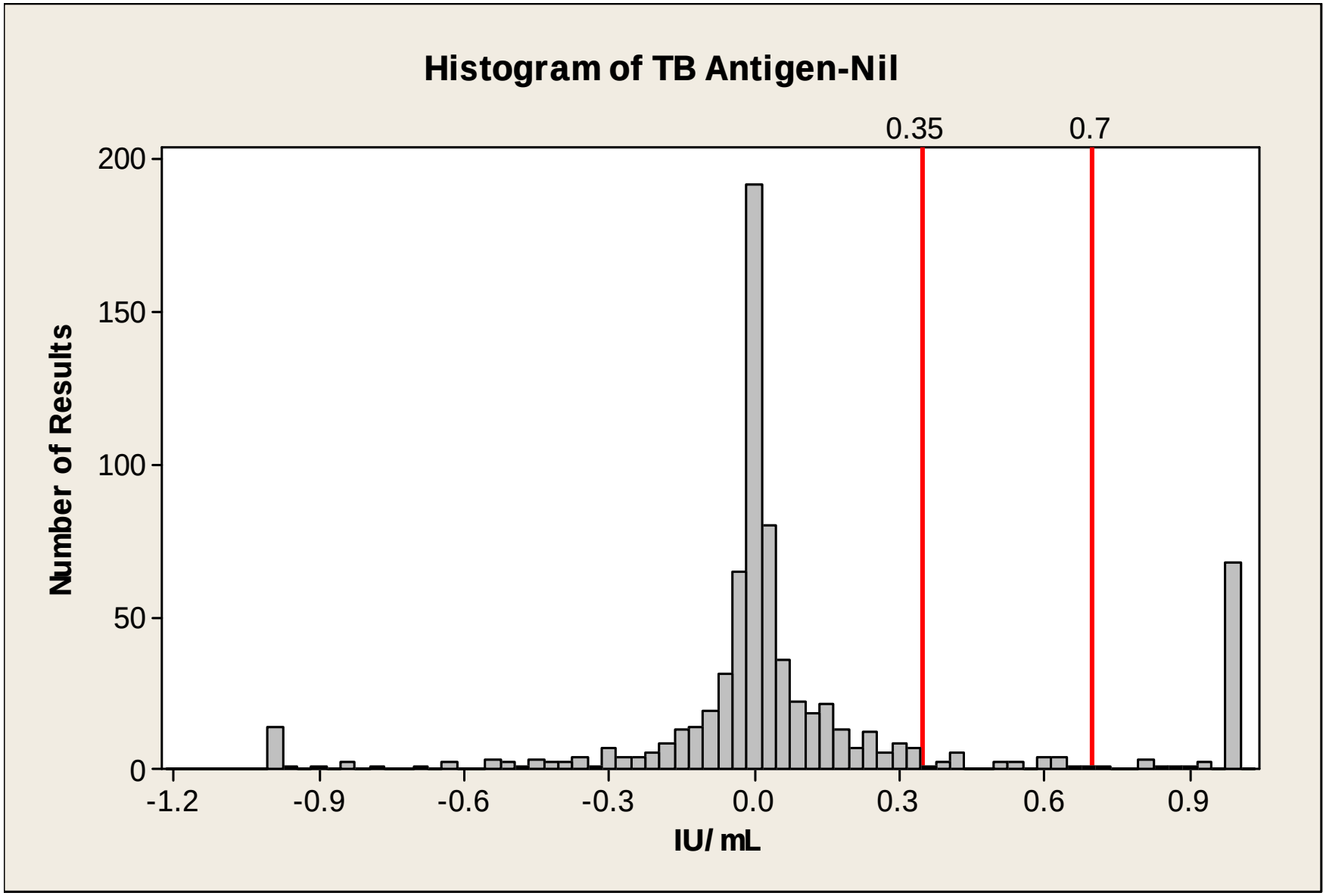

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Comparison of IGRAs

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

*32 hours, if use T-Cell *Xtend* Reagent (not FDA-cleared)

Advantages of IGRAs

- Requires single patient visit
- Results available within 24 h
- Not subject to reader interpretation
- Not affected by prior BCG vaccination
- May reduce number of X-rays
- May reduce INH usage and resulting liver toxicity
- More sensitive and specific than TST

MMWR, 59,RR-5, June 25, 2010

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Disadvantages of IGRAs

- Blood samples must be processed within 16 h (QFT) or within 8/32 h (TB-Spot) after draw
- QFT needs vendor-supplied transport tubes
- T-Spot technically complex
- Don’t differentiate between active/latent TB
- Limited data in
  - Children
  - Immunocompromised (AIDS, etc.)—not recommended for routine use in HIV pediatric patients (MMWR, 2009, 58, (RR11: 1-166))

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