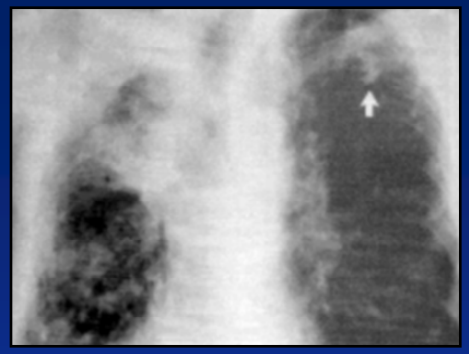

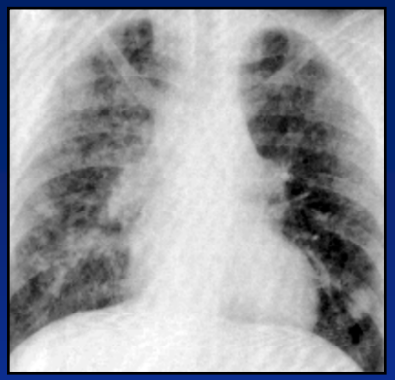

IGRAs in Children

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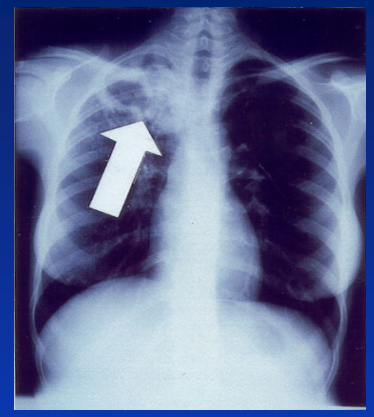
Disclosure Statement: Deborah Lewinsohn

- I have no significant financial interests in Cellestis or Oxford Immunotech.
- All relevant financial interests are as follows:
  - Cellestis: Honoraria for three Cellestis-sponsored meetings for role as a participant, moderator, or invited speaker.
  - Oxford Immunotech: Sponsored research contract to provide T cells to use for QC for T-spot.*TB*.

Natural History of *M. tuberculosis* Infection



1° Progressive Disease (<5% vs. 50-80%)

Reactivation Disease (5-10%)

Persistent Infection (90% vs 5-50%)

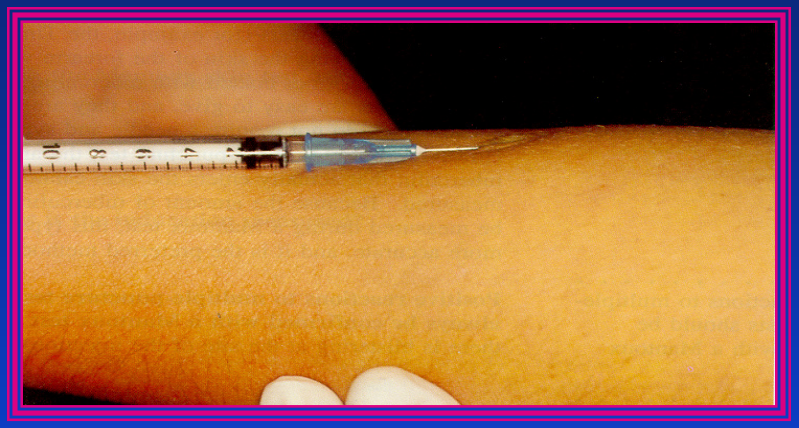
Bacterial Load

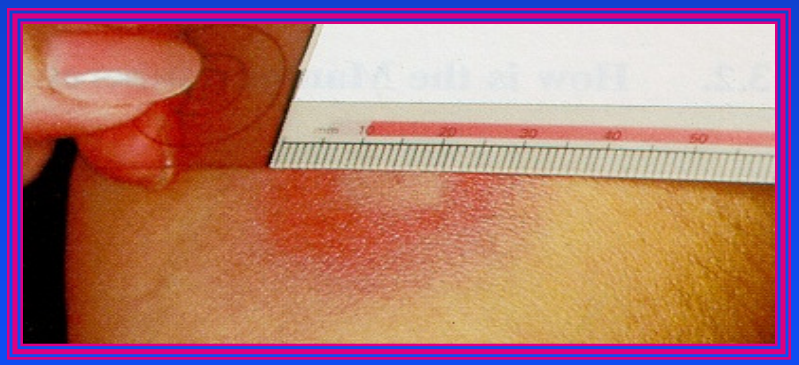
Innate (TST-)

Adaptive (TST+)

Adapted from: Henry Boom, TBRU, CWRU

Diagnosis of LTBI using TST



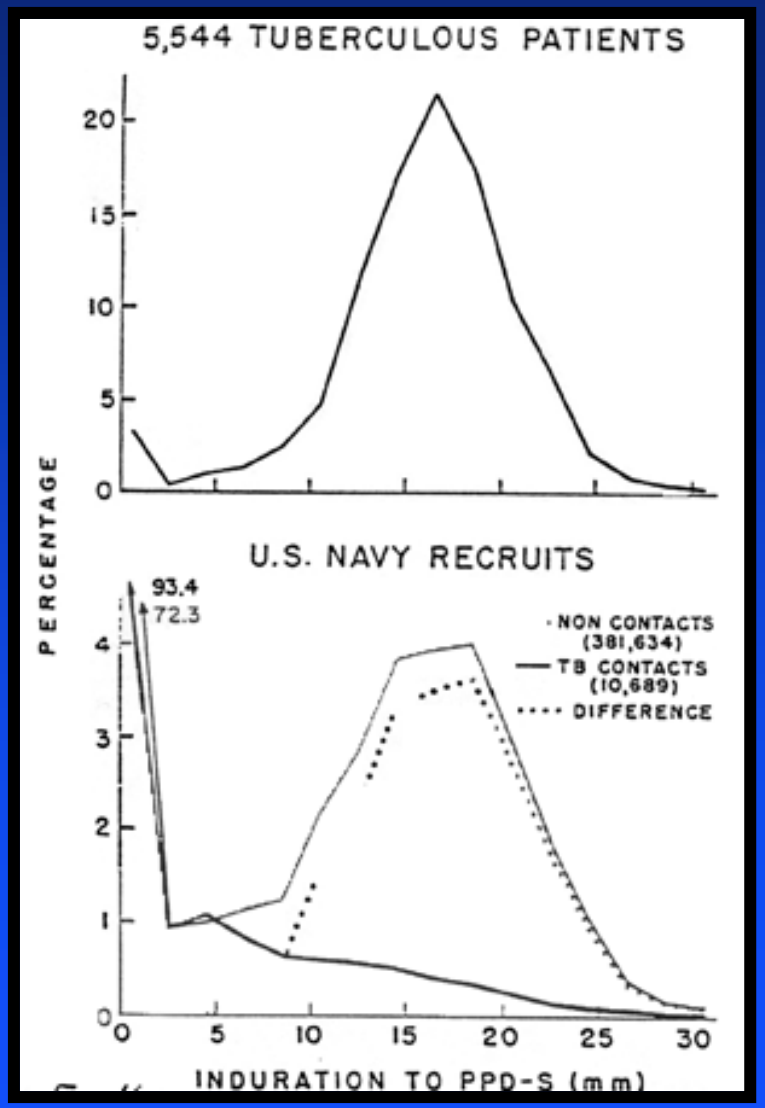


Photographs: Dr. Chuck Daley, National Jewish Medical Center

Problems include:

- Inter-reader variability; requires return visit.
- Confusing cutoffs for various risk groups.
- Low positive predictive value in countries with low prevalence
- False negatives
  - Anergy: HIV/ESRD
  - Recent TB
  - Very young
  - Overwhelming disease
- False positives
  - BCG vaccination
  - Nontuberculous mycobacteria

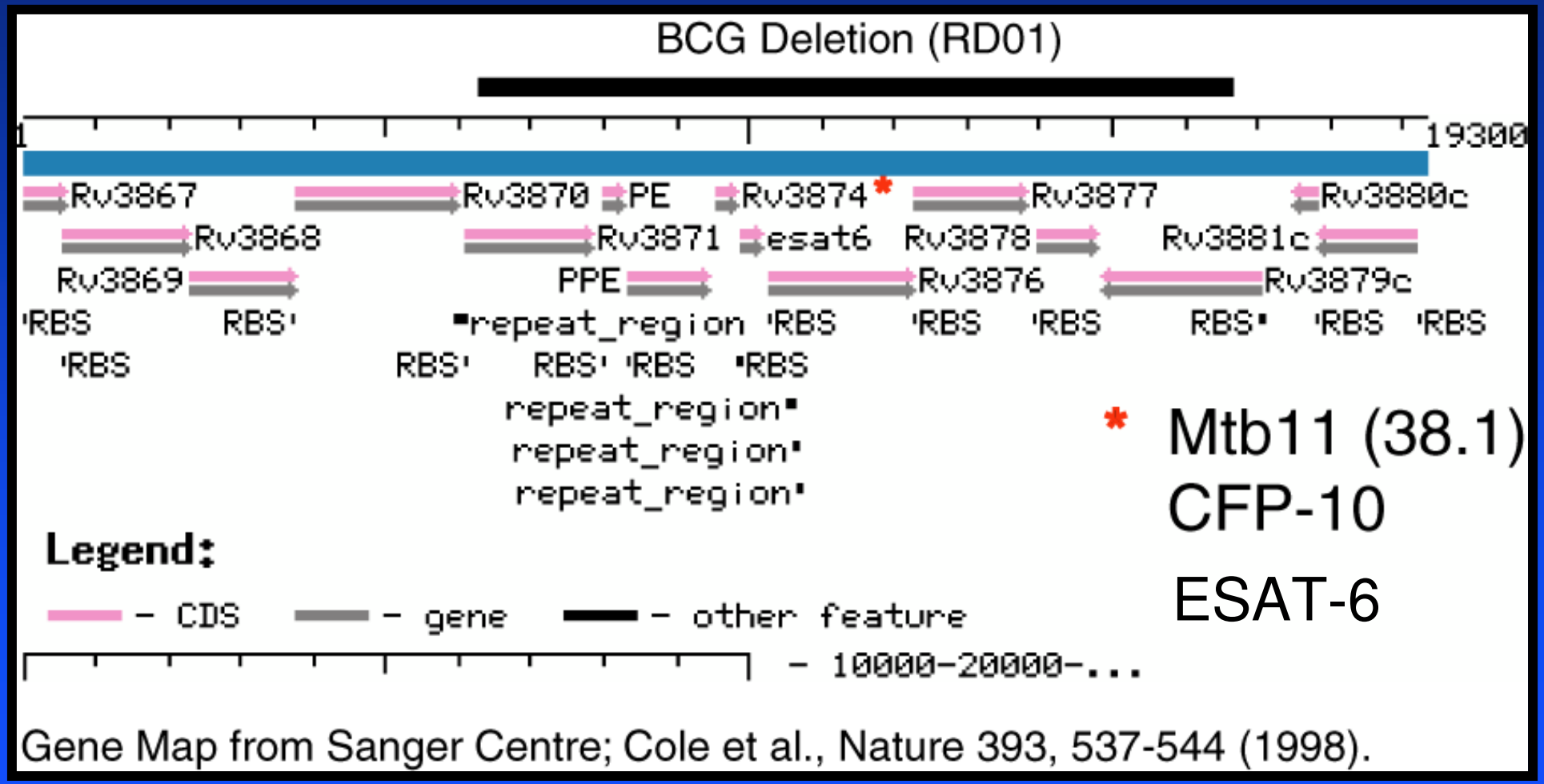
Diagnosis of Mtb infection by TST: TB controllers' comfort food



T cell based diagnostics for TB: IFN-γ Release Assays (IGRA's)

- Potent pro-inflammatory cytokine released by T cells and NK cells. Assays reflect adaptive T cell response to TB.
- Two commercially available tests:
  - T-spot®.*TB*; ELISPOT.
  - QuantiFERON®-TB Gold IT; ELISA.

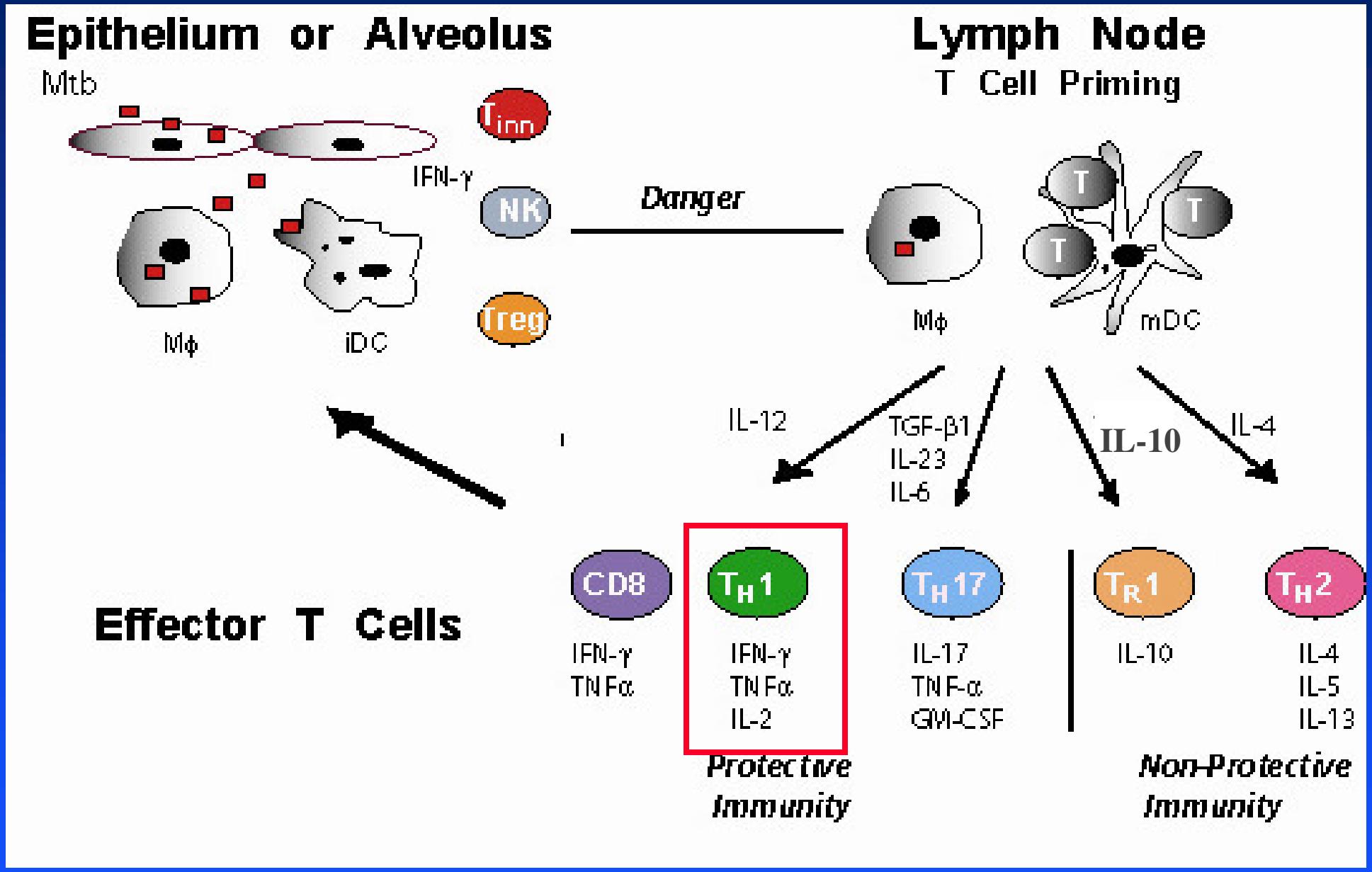
Antigens Absent from BCG



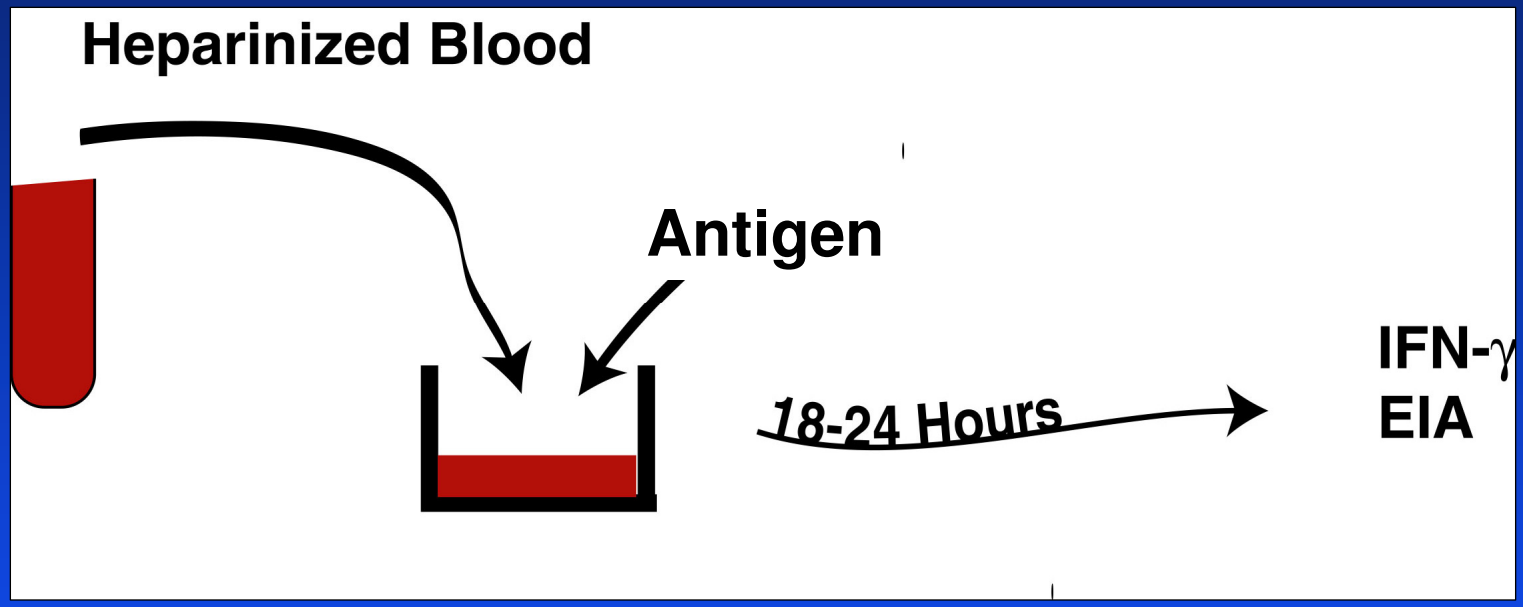
Gene Map from Sanger Centre; Cole et al., Nature 393, 537-544 (1998).

TST vs IGRA

	TST	IFN
Cell Types	CD4 CD8 Basophils DC and Macrophages	CD4 (CD8)
Cytokines	IL-4, IFN-γ TNF-α, IL-10, IL-12, G-CSF,	IFN-γ
Associated with Protective Immunity	No	Yes
Timing	2-5 Days	Short Term
Homing Phenotype	Skin	Polymorphic


Epithelium or Alveolus
Lymph Node
T Cell Priming
Mtb
IFN-γ
Mϕ
iDC
Danger
Mϕ
mDC
T
T
T
IL-12
TGF-β1
IL-23
IL-6
IL-10
IL-4
Effector T Cells
CD8
IFN-γ
TNFα
TH1
IFN-γ
TNFα
IL-2
TH17
IL-17
TNF-α
GM-CSF
TR1
IL-10
TH2
IL-4
IL-5
IL-13
Protective Immunity
Non-Protective Immunity

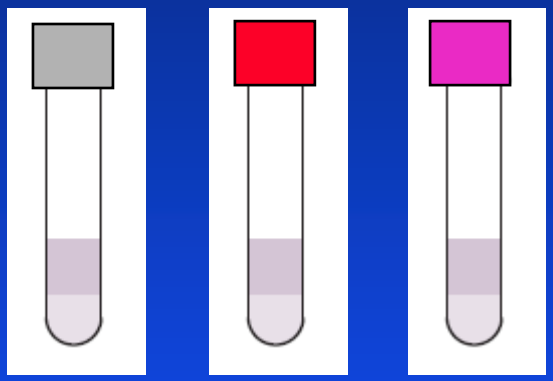
QuantiFERON-TB PPD vs. QuantiFERON-TB Gold



QFT-TB PPD (1st)	QFT-TB Gold (2nd)
PPD	ESAT-6
M. Avium control	CFP10
Mitogen Control	Mitogen

QuantiFERON®-TB Gold IT: ELISA

1 ml 1 ml 1 ml

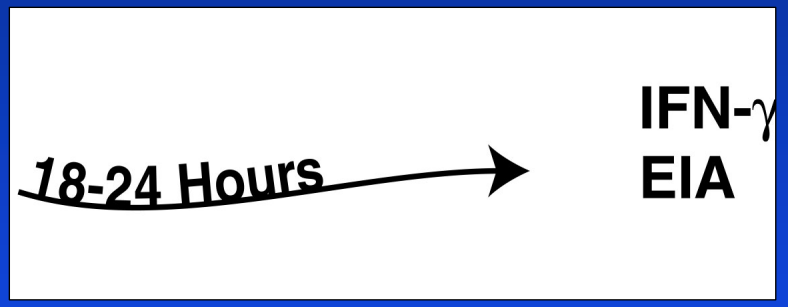


Nil **TB Antigens** **Mitogen**

CFP-10
ESAT-6
TB7.7

Transport

< 16 hours at room temperature



Incubation at 37°

Adapted from QuantiFERON®-TB Gold IT product insert

QuantiFERON®-TB Gold IT: ELISA
Interpretation of test result

Nil [IU/mL]	TB Antigen minus Nil [IU/mL]	Mitogen minus Nil [IU/mL][1]	QuantiFERON®-TB [IU/mL]	Report/Interpretation
≤ 8.0	< 0.35	≥ 0.5	**Negative**	*M. tuberculosis* infection NOT likely
	≥ 0.35 and < 25% of Nil value	≥ 0.5		
	≥ 0.35 and ≥ 25% of Nil value	Any	**Positive**[2]	*M. tuberculosis* infection likely
	< 0.35	< 0.5	**Indeterminate**[3]	Results are indeterminate for TB-Antigen responsiveness
	≥ 0.35 and < 25% of Nil value	< 0.5		
> 8.0[4]	Any	Any		

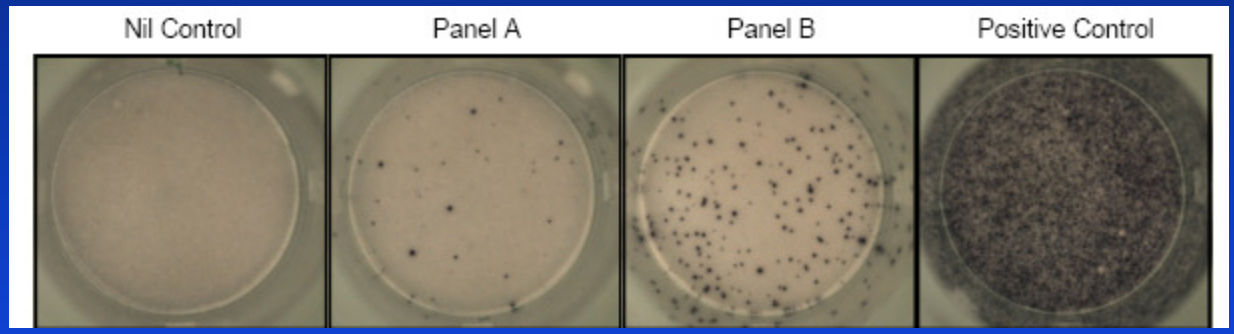
From: QuantiFERON®-TB Gold IT product insert

T-spot®.*TB*: ELISPOT

CPT tubes

Transport < 8 hr at room temperature

ELISPOT assay



- Adults and children 10 years old and over: one 8mL or two 4mL tubes
- Children 2-9 years old: one 4mL tube
- Children up to 2 years old: 2mL paediatric tube

From: T-spot®.TB Visual procedure guide

T-spot®.*TB*: ELISPOT
Interpretation of test result

- The test result is Positive if (Panel A-Nil) and/or (Panel B-Nil) ≥ 8 spots.
- The test result is Negative if both (Panel A-Nil) and (Panel B-Nil) ≤ 4 spots.
- The test result is Borderline if the highest of the Panel A or Panel B spot count (minus Nil) is 5, 6 or 7.
- The test result is Invalid if:
  - Nil > 10 spots.
  - Mitogen < 20 spots AND both (Panel A-Nil0 and (Panel B-Nil) ≤ 4 spots.

From: Summarized from T-spot®.TB Product insert

Use of IGRA's in adults

- CDC Recommendations: "QFT-G can be used in all circumstances in which the TST is used, including contact investigations, evaluation of recent immigrants who have had BCG vaccination, and TB screening of health-care workers and others undergoing serial investigation for *Mycobacterium tuberculosis* infection. QFT-G usually can be used in place of (and not in addition to the TST)" (MMWR, Dec. 16, 2005, Vol.54.)
- FDA Approvals:
  - QFT TB (11/28/01); QFT TB Gold (12/2/04); QFT TB Gold-IT (10/10/07)
  - T-SPOT.*TB* (7/25/08)

Evidence-based evaluation of IGRA's vs TST

Evaluation without reference to a gold standard diagnostic for LTBI:

- Sensitivity in culture positive TB.
- Relationship with exposure risk factors.
- Specificity in low risk populations.
- Prospective prognostic studies.

Evaluation vs TST in children: Promise of IGRA's

Evaluation without reference to a gold standard diagnostic for LTBI:

- **Sensitivity in culture positive TB.**
- Relationship with exposure risk factors.
- Specificity in low risk populations.
- Prospective prognostic studies.

QFT-Gold for Diagnosis of LTBI in active TB

QFT version	Study, year, country	Age range	Young/total	Sensitivity of TST in active TB n/N (%)	Sensitivity of IGRA in active TB n/N (%)
QFT Gold	Connell, 2006, Australia	0 - 18	NR/101	9/9 (100)	9/9 (100)
QFT Gold	Okada, 2007, Cambodia	0 - 5	210/210 < 6 yrs	15/19 (79)	10/19 (53)
QFT Gold IT	Connell, 2008, Australia	1 - 19	NR/100	NR	8/9 (89)
QFT Gold IT	Detjen, 2007, Germany	0 - 16	54% < 3 yrs; n = 73	28 /28 (100)	26/28 (93)
QFT Gold IT	Dogra, 2007, India	1 - 12	42/105 (40%) < 5 yrs	5/8 (52) –Cx+ 9/11 (82)-Rx	5/8 (52) – Cx+ 9/11 (82) - Rx
QFT Gold IT	Dominguez, 2007, Spain	0 - 18	15/134 (11%) < 5 yrs	9/9 (100)	6/9 (67)
QFT Gold IT	Kampmann, 2009, UK	2 - 16	26/91 < 5 years	20/24 (83) Cx+	20/25 (80) Cx+

T-spot®.*TB* for Diagnosis of LTBI in active TB

Study, year, country	Age range	Young/total	Sensitivity of TST in active TB n/N (%)	Sensitivity of IGRA in active TB n/N (%)
Connell, 2008, Australia	1 - 19	NR/101	NR	9/9 (89)
Detjen, 2007, Germany	0 - 16	54% < 3 yrs; n = 73	28 /28 (100)	26/28 (93)
Dominguez, 2007, Spain	0 - 18	15/134 (11%) < 5 yrs	9/9 (100)	6/9 (67)
Kampmann, 2009, UK	2 - 16	26/91 < 5 years	20/24 (83)	14/24 (58)
Nicol, 2009, S. Africa	0 - NR	204/214 < 3 years	30/58 (52)	23/58 (40)
Warier, 2009 India	0 - 18	NR/15	NR	8/15 (53) – Cx+

Evaluation vs TST in children: Promise of IGRA's

Evaluation without reference to a gold standard diagnostic for LTBI:

- Sensitivity in culture positive TB.
- **Relationship with exposure risk factors.**
- Specificity in low risk populations.
- Prospective prognostic studies.

QFT-Gold for LTBI diagnosis

QFT version	Study, year, country	Age range	Young/total	TST positivity rate n/N (%)	Concordance TST/IGRA
QFT Gold	Connell, 2006, Australia	0 - 18	NR/101	42/42 (100)	11/42 (26); K = 0.30; TST+/QFT-
QFT Gold	Hesseling, 2008, S. Africa	0 - 5	29/29	15/28 (54)	NR (88.9); K = 0.78; TST+/QFT-
QFT Gold	Mandalakas, 2008, S. Africa	NR, X = 4.4	All HIV+; NR/23	6/23 (26)	NR (75) K = .44; TST+/QFT-
QFT Gold	Okada, 2007, Cambodia	0 - 5	210/210 : < 6 yrs	47/195 (24)	171/195 (88); K = 0.62; TST+/QFT-
QFT Gold IT	Chun, 2008, Korea	0-13	NR: Med = 1.7 yr	26/42 (62)	24/42 (57); K = 0.19
QFT Gold IT	Connell, 2008, Australia	1 - 19	NR/100	47/97 (48)	NR; (75); K = .5
QFT Gold IT	Lighter, 2008, USA	0 - 18	67/207, < 5 yrs	116/204 (56)	112/207 (55); K = .17 TST+/QFT-
QFT Gold IT	Nakaoka,2006, Nigeria	0 - 14	NR/207	57/206 (28)	49/66 (74); K = .74; TST-/QFT+
QFT Gold IT	Dogra, 2007, India	1 - 12	42/105 , < 5 yrs	10/105 (9.5)	100/105; (95); K = .73
QFT Gold IT	Dominguez, 2007, Spain	0 - 18	15/134 (11%)	115/134 (86)	BCG-:23/40 (58), K=.24
QFT Gold IT	Kampmann,2009, UK	0-16	56/118; < 5 yrs	57/114 (50)	NR (77); K = .53
QFT Gold IT	Tsiouris, 2006, South Africa	5-15	NA/NR	80/184 (44)	145/184 (79); K=.56; TST+/QFT-

Diagnosis of LTBI/household contacts: QFT

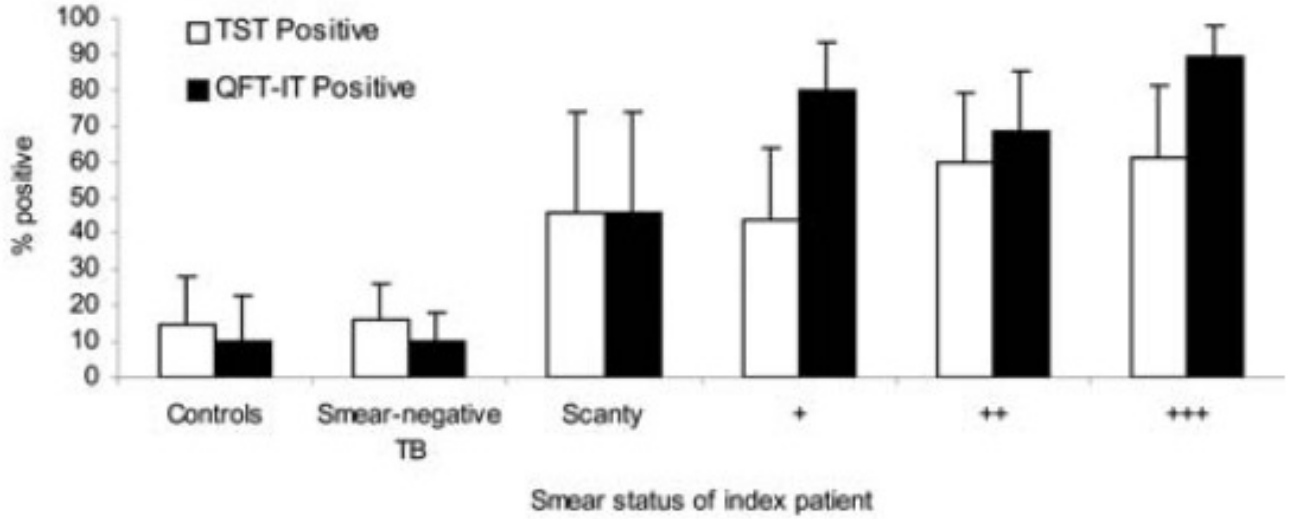


Figure 2. Proportion of children with positive tuberculin skin test (TST) (>10 mm) and QuantiFERON Gold in Tube (QFT-IT) test results, by adult smear positivity. Error bars show 95% confidence intervals.

QFT-TB Gold-IT, Nigeria
PPD Chiron (5 IU): positive = ≥ 10 mm

Nakaoka, H., Emerg Inf Dis, 2006

T-spot®.*TB* for LTBI diagnosis

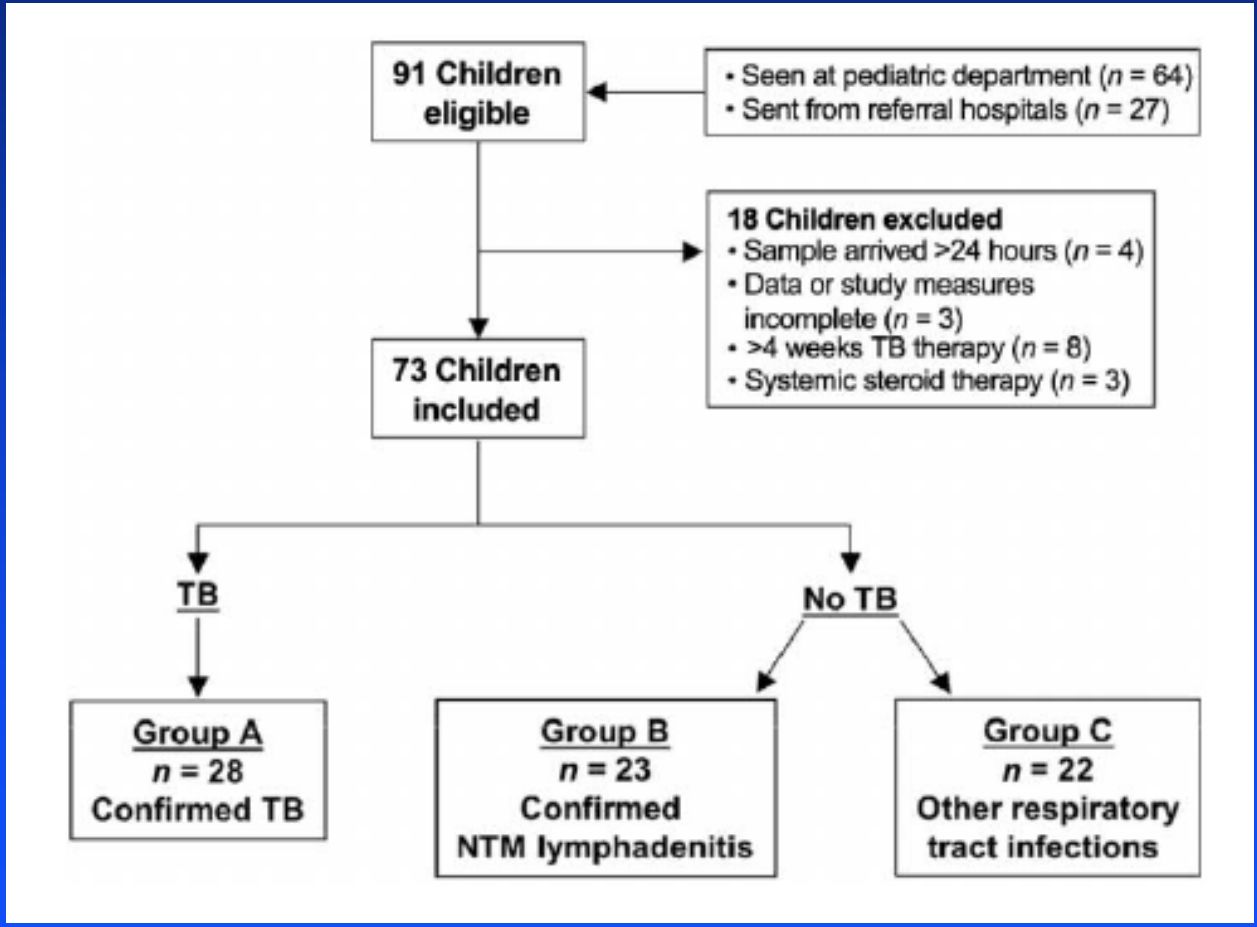
Study, year, country	Age range	Young/total	TST positivity rate n/N (%)	Concordance TST/QFT
Connell, 2008, Australia	1 - 19	NR/100	47/97 (48)	75% K = .51
Dominguez, 2007, Spain	0 - 18	15/134 (11%)	115/134 (86)	BCG-:25/40 (62), K=.33
Hesseling, 2008, South Africa	0-5	29/29	15/28 (54)	NR (46), K=-0.15 TST-/IGRA+
Kampmann, 2009, UK	0-16	56/118; < 5 yrs	57/114 (50)	NR (75); K = .49
Mandalakas, 2008, S. Africa	NR, X = 4.4	All HIV+; NR/23	6/23 (26)	NR (67) K = .33; TST-/IGRA+

Evaluation vs TST in children: Promise of IGRA's

Evaluation without reference to a gold standard diagnostic for LTBI:

- Sensitivity in culture positive TB.
- Relationship with exposure risk factors.
- **Specificity in low risk populations.**
- Prospective prognostic studies.

Specificity of Diagnosis of LTBI in TB suspects: QFT-IT/ T-spot®.*TB* vs TST



QFT-TB Gold-IT vs T-spot, Germany
PPD Chiron (10 TU), positive ≥ 5 mm

Detjen, et al., CID, 2007.

Specificity of Diagnosis of LTBI in TB suspects: QFT-IT/ T-spot®.*TB* vs TST

Table 3. Indicators of diagnostic accuracy for the tuberculin skin test (TST), QFT-IT, T-SPOT, and the combination of QFT-IT and T-SPOT.

Test	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)	Positive PV, % (95% CI)	Negative PV, % (95% CI)
TST	100 (88–100)	58 (42–73)	2.4 (1.6–3.4)	0.1[a] (0.01–0.4)	62 (47–76)	100 (85–100)
QFT-IT	93 (77–99)	100 (91–100)	37.1[b] (5.3–258.0)	0.1 (0.02–0.3)	100 (87–100)	95 (84–99)
T-SPOT	93 (77–99)	98 (87–100)	37.1 (5.3–258.0)	0.1 (0.02–0.3)	96 (81–100)	95 (83–99)
QFT-IT and T-SPOT (both positive)	89 (72–98)	100 (91–100)	35.7[b] (5.1–248.4)	0.1 (0.04–0.3)	100 (86–100)	93 (81–99)
QFT-IT and T-SPOT (1 positive)	96 (82–100)	98 (87–100)	38.6 (5.6–267.5)	0.04 (0.01–0.3)	96 (82–100)	98 (87–100)
Stepwise approach[c]	93 (77–99)	100 (91–100)	37.1[b] (5.3–258.0)	0.1 (0.02–0.3)	100 (87–100)	95 (84–99)

NOTE. Calculations were based on 68 children, including 28 children with confirmed tuberculosis (group A) and 40 unvaccinated children without tuberculosis (19 children with confirmed nontuberculous mycobacterial lymphadenitis [group B] and 21 children with other respiratory tract infections [group C]). LR, likelihood ratio; PV, predictive value; QFT-IT, QuantiFERON-TB Gold In-Tube (Cellestis); T-SPOT, T SPOT-TB (Oxford Immunotec).

[a] For calculation of this LR, we classified 1 correct diagnosis in a child with tuberculosis as a false-negative result (to avoid division by 0).

[b] For calculation of this LR, we classified 1 correct diagnosis in a child without tuberculosis as a false-positive result (to avoid division by 0).

[c] We also calculated diagnostic accuracy in a stepwise approach according to recent National Institute of Health and Clinical Excellence guidelines [15]; only patients with positive TST results were tested with IFN-γ release assays. QFT-IT and T-SPOT showed the same values for all indicators.

QFT-TB Gold-IT vs T-spot, Germany
PPD Chiron (10 TU), positive ≥ 5 mm

Detjen, et al., CID, 2007.

Specificity of IGRA in BCG-vaccinated children

- Inclusion: No risk and TST ≥ 5 mm.
- 62 children age 2 months – 14 years, all BCG vaccinated.
- 0/62 QFT-IT positive

QFT-IT, Korea
PPD RT 23 (2 TU), positive ≥ 5 mm induration

Chun, et al., Diag Micro Inf Dis, 2008

Evaluation vs TST in children: Promise of IGRA's

Evaluation without reference to a gold standard diagnostic for LTBI:

- Sensitivity in culture positive TB.
- Relationship with exposure risk factors.
- Specificity in low risk populations.
- **Prospective prognostic studies.**

Diagnosis of LTBI/school contacts: QFT

- 349 15-16 y/o boys, all BCG vaccinated.
- Tested with TST: 95 of 349 positive.
- 88 TST positive tested with QFT-TB Gold: 4 of 88 positive.
- 3 of 4 in high exposure group – received INH.
- Remaining TST positive students – no INH and no disease with 3+ years follow-up.

QFT-TB Gold, Japan
PPD Nippon (≈ 2.5 TU), positive ≥ 30 mm erythema

Higuchi, K., et al., Respirology, 2007

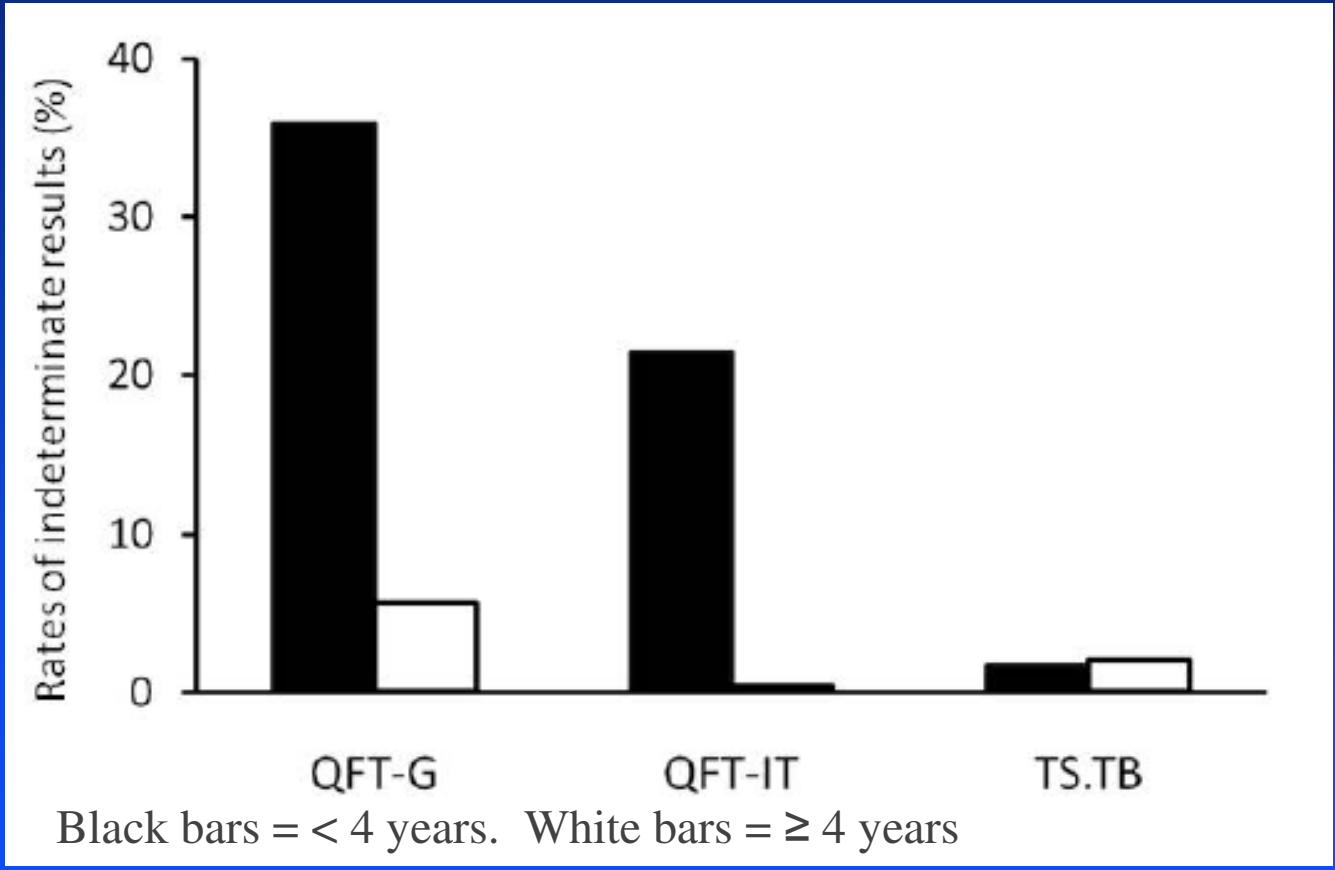
Limitations of IGRA's in Children

- Paucity of data in children < 5 years.
- Increased frequency of indeterminate assays in children < 5 years.
- Required blood volumes.
- Lack of longitudinal data.

Limitations of IGRA's in young children

QFT version	Study, year, country	Age range	Young/total	Indeterminate n/N (%)	QNS Blood n/N (%)	
QFT Gold	Connell, 2006, Australia	0 - 18	NR/101	17/101 (17)	3/101 (3)	
QFT Gold	Hesseling, 2008, S. Africa	0 - 5	29/29	3/21 (14)	7/29 (24)	
QFT Gold	Mandalakas, 2008, S. Africa	NR, X = 4.4	All HIV+; NR/23	0/12 (0)	11/23 (47)	
QFT Gold	Okada, 2007, Cambodia	0 - 5	210/210 : < 6 yrs	9/208 (6)	13/217 (6)	
QFT Gold IT	Bruzzese, 2009, Italy	2 - 24	NR; all HIV neg, immunocomp	16/80 (20)	NR	
QFT Gold IT	Chun, 2008, Korea	0-13	NR: Med = 1.7 yr	17/227 (7.5)	NR	
QFT Gold IT	Connell, 2008, Australia	1 - 19	NR/100	0/38 (0)	5/101 (5)	
QFT Gold IT	Lighter, 2008, USA	0 - 18	67/207, < 5 yrs	3/207 (1)	0/207 (0)	
QFT Gold IT	Nakaoka,2006, Nigeria	0 - 14	NR/207	33/207 (16)	NR	
QFT Gold IT	Dogra, 2007, India	1 - 12	42/105 , < 5 yrs	0/105 (0)	0/105 (0)	
QFT Gold IT	Dominguez, 2007, Spain	0 - 18	15/134 (11%)	3/134 (2)	NR	
QFT Gold IT	Kampmann,2009, UK	0-16	56/118; < 5 yrs	14/209 (7)	0/209 (0)	
QFT Gold IT	Tsiouris, 2006, South Africa	5-15	NA/NR	NR	37/221 (17)	

Indeterminate rates of IGRA's

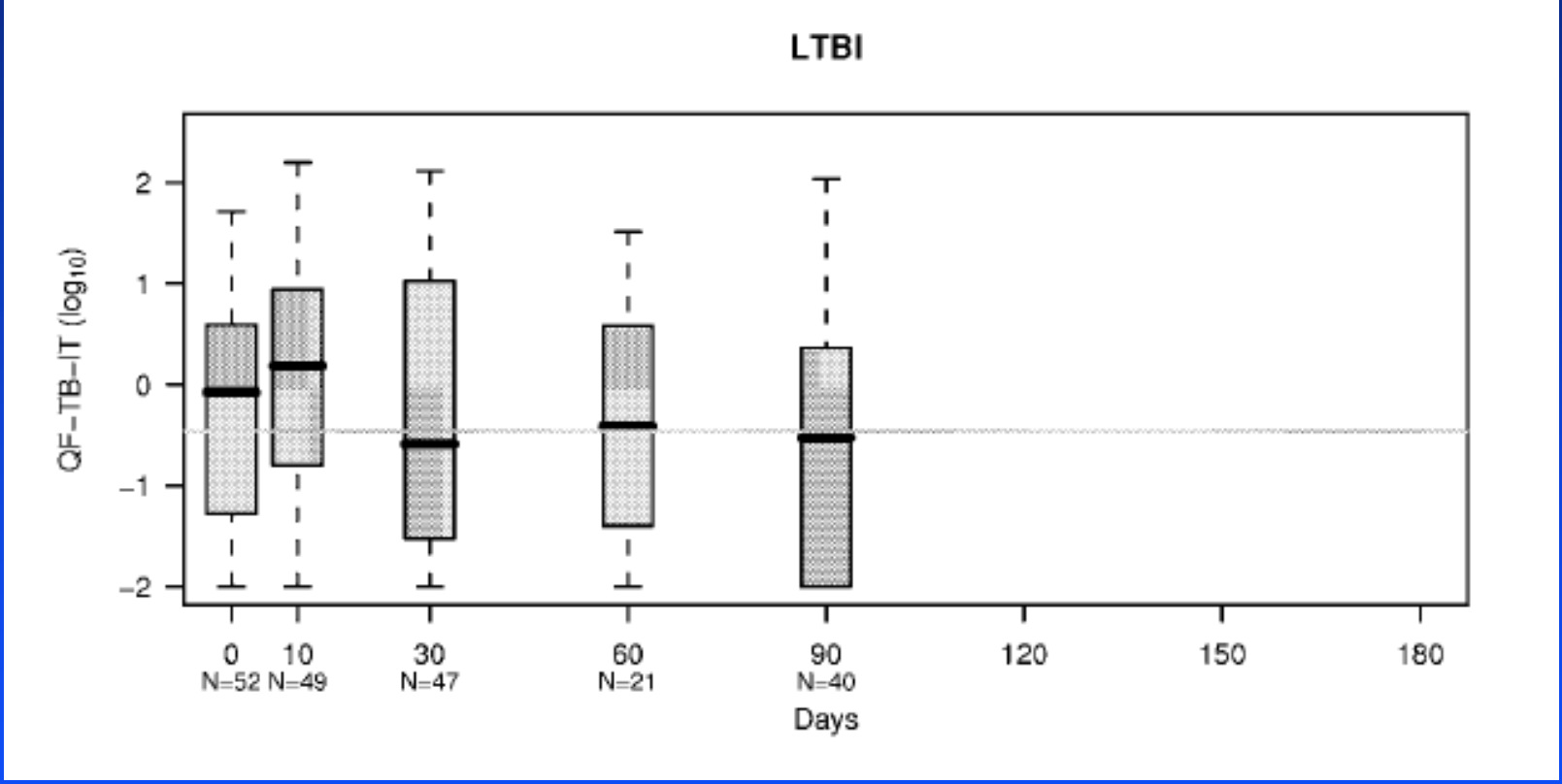


Black bars = < 4 years. White bars = ≥ 4 years

QFT-TB Gold vs QFT-IT vs T-spot, Italy

Bergamini, et al., Pediatrics, 2009.

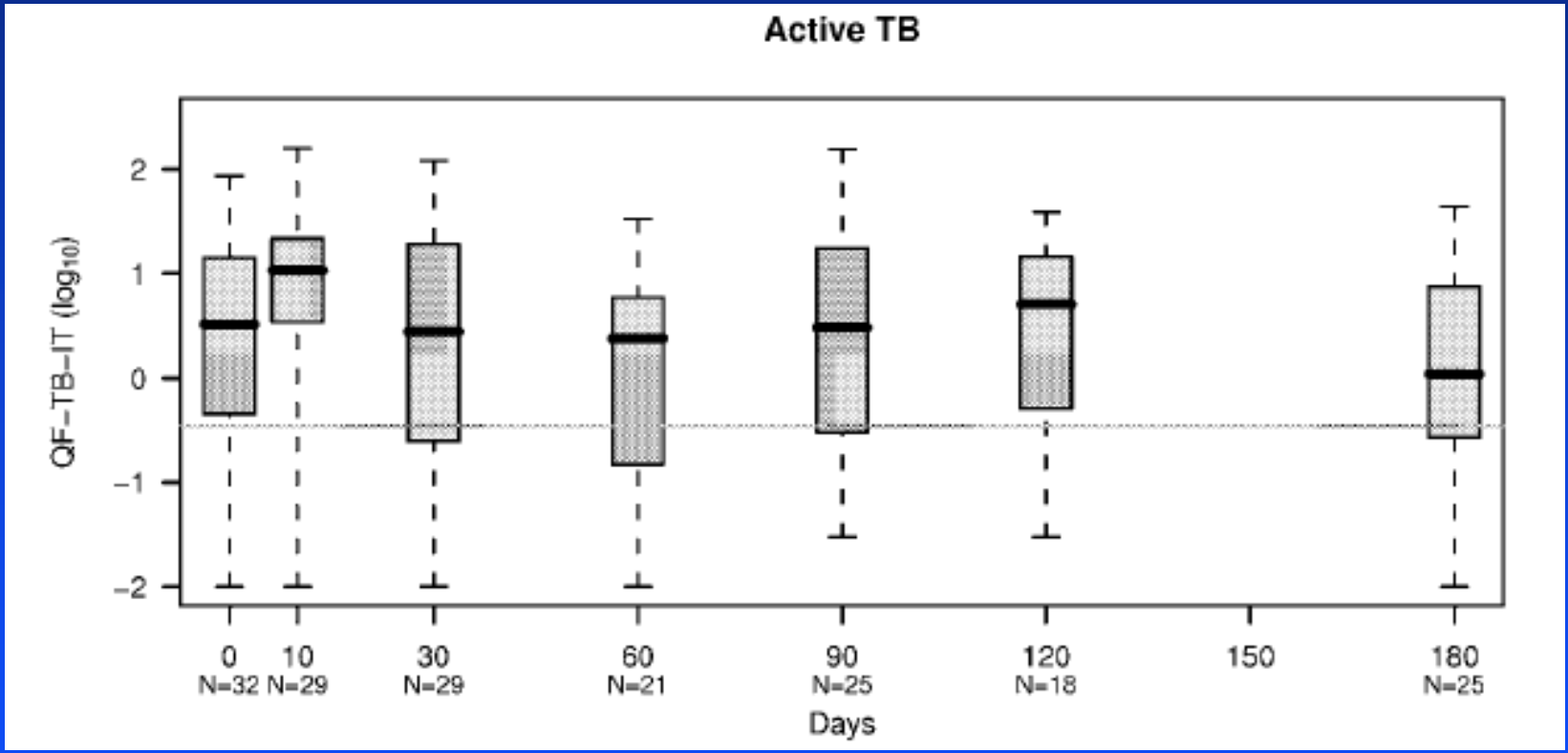
Longitudinal IGRA data in children



QFT-IT
France

Herrmann et al., PLoS ONE, 2009.

Longitudinal IGRA data in children



QFT-IT
France

Herrmann et al., PLoS ONE, 2009.

QFT vs T-SPOT.*TB*

Specificity: QFT

- BCG-vaccinees

Sensitivity: T-SPOT.*TB*

- Immunocompromised
- Young children

What would we like in a test?

Specificity and Sensitivity: The DiCaprinator

With permission: Photoshop by David Lewinsohn

QFT vs T-SPOT.*TB* in children

- QFT more available than T-SPOT.*TB*
- More published data in children for QFT than for T-SPOT.*TB*.
- Less indeterminate results for T-SPOT.*TB* compared to QFT.
- Less blood required for T-SPOT.*TB* than for QFT.
- Specificity equivalent in one study.
- Sensitivity of T-SPOT.*TB* increased, equal, decreased when compared with QFT-IT.
- Increase positive tests with T-SPOT.*TB*

Indications for IGRA's and TST in children (My opinion)

- Close contacts of active TB cases:
- Immigrants from endemic countries:
- Significant travel history:
  - IGRA preferred to TST in children ≥ 5 years.
  - TST preferred to IGRA in children < 5 years.

Indications for IGRA's and TST in children (My opinion - continued)

- TB suspects:
- HIV infection:
- Increased risk of progression of LTBI:
  - Consider both IGRA and TST and take either positive as evidence of infection.

Upcoming recommendations

- ATS/CDC/IDSA (AAP) guidelines:
- CDC guidelines – updated for QFT-Gold-IT and T-SPOT.*TB*.
- AAP – 2009 RedBook

Future research needs for IGRA's in children

- High risk young children in low incidence setting (Household contact study in young children).
- Longitudinal data in young children (Data to inform "window prophylaxis").
- Young immunosuppressed and HIV-infected children.

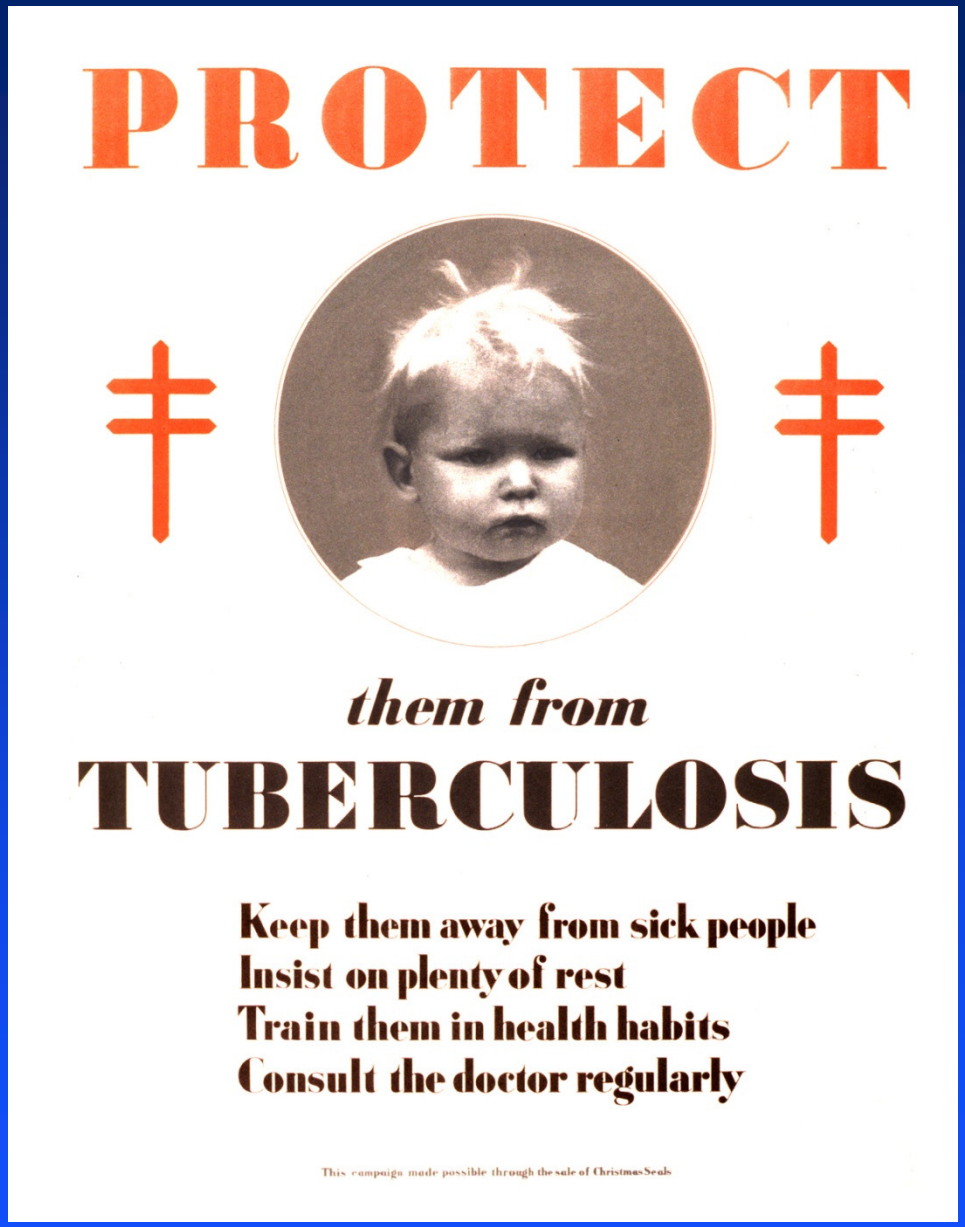
New assays for TB diagnosis in children

- IP-10 assays (ELISA/CD4)
- IFN-γ , IL-2, TNF-α (Flow cytometry)
- CD8+ T cells (ELISPOT)
- Antibodies in lymphocyte supernatant (ALS)

Slides Credits:

Henry Boom

Chuck Daley

David Lewinsohn

Madhu Pai