

Field evaluations of IFN- γ assays in rural India



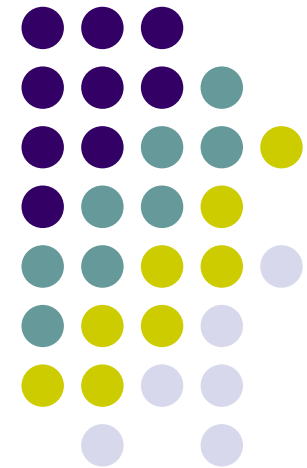
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TB in India

- Estimated 40% LTBI prevalence (?)
- 2 million cases/year
- Estimated ARI: 1.5%
- High BCG coverage
- Widespread NTM exposure

IGRA studies

- HCWs (cross-section and serial testing)
- Children
- Active TB (pre and post-treatment)
- Pre and post LTBI therapy
- Household contacts



Outline



- Discordance, cut-points, and analyses of continuous outcomes
- Serial IGRA testing: conversions and reversions
- T cell responses after anti-TB therapy
- T cell responses after isoniazid preventive therapy

Discordance, cut-points, continuous outcomes



- 10 – 40% discordance in various studies
- Discordance analysis is helpful, particularly since there is no gold standard
- Tendency to assume that IGRA results are more likely to be “true” and, discordance is because of false TST results
- This approach is simplistic and ignores the fact that TST performs quite well in certain populations
 - TST specificity is fairly high in populations where BCG is given during infancy (e.g. India) [Menzies 2000; Farhat M et al. Unpublished]
- While some discordance (TST+/IGRA-) may be due to BCG in certain studies (especially if BCG given after infancy), other studies found no clear explanations for discordance

Discordance, cut-points, continuous outcomes



- **Extreme discordance** reported; the biological basis needs to be studied
- For example, among those with TST ≥ 15 mm
 - Mahomed (IJTLD 2006): 31% were negative by QFT-G *In Tube*
 - Ferrara (AJRCCM 2005): 43% were negative by QFT-G
 - Pai (JAMA 2005): 11% were negative by the QFT-G *In Tube*
 - Could be false-positive TST
 - What exactly are IGRAs measuring?
 - a subset of LTBI (i.e. those with recent, persistent infection versus remote infection which has been resolved spontaneously or been treated?)
- Discordance of the reverse type (i.e. TST-/IGRA+) has been frequently documented; this phenomenon is largely unexplained
 - For example, among those with a TST of 0 mm:
 - Pai (JAMA 2005), found 12% positive by QFT-G *In Tube*
 - Ferrara (AJRCCM 2005) found 9% positive by QFT-G
 - ? exposure to certain NTM species that do contain RD1 antigens

Cut-points & continuous outcomes



- Most studies have analyzed both TST and IGRA results as dichotomous (yes/no) outcomes
- This approach ignores the fact that both tests produce measures that are inherently continuous
- Cut-points can affect concordance
- Cut-points may also partially explain differences across studies

		QFT+	QFT-		Agreement (k)
TST results (10mm)	TST+	226	72	298	81.4 (0.61)
	TST-	62	359	421	
		288	431	719	

Effect of cut-points on concordance (agreement)



	QFT-G In Tube cut-points (IFN-g IU/mL)								
		0.10	0.20	0.30	0.35**	0.40	0.50	0.60	0.70
TST Cut-point	5 mm	71.3	71.7	71.8	71.4	70.8	70.4	70.1	70.1
	6 mm	73.5	74.4	75.1	74.7	74.7	74.3	74.0	74.0
	7 mm	75.0	77.1	77.8	77.5	77.4	76.9	76.9	76.9
	8 mm	76.1	78.8	79.7	79.4	79.3	78.9	79.2	79.2
	9 mm	75.9	79.4	80.4	80.1	80.3	79.9	80.1	80.1
	10 mm	75.6	79.9	81.7	81.4	81.8	81.4	81.9	82.2
	11 mm	75.3	80.4	82.8	82.5	83.2	82.8	83.3	83.6
	12 mm	74.7	80.4	82.8	82.5	83.2	83.1	83.6	84.2
	13 mm	73.8	79.7	82.1	81.8	82.5	82.6	83.2	83.8
	14 mm	71.5	77.5	80.1	80.1	80.8	81.3	82.4	83.5
	15 mm	68.2	74.4	77.9	77.9	78.6	79.0	80.7	81.8

Variability around cut-points



- Of the total of 134 discordant results:
 - 50 (37%) had TST induration sizes within 2 mm of the cut-point of 10 mm [could be partly digit preference]
 - 28 (21%) had IFN-g values within 0.20 IU/mL of the QFT-G cut-point of 0.35 IU/mL.

		QFT+	QFT-		Agreement (k)
TST results (10mm)	TST+	226	72	298	81.4 (0.61)
	TST-	62	359	421	
		288	431	719	

Framework for discordance analyses



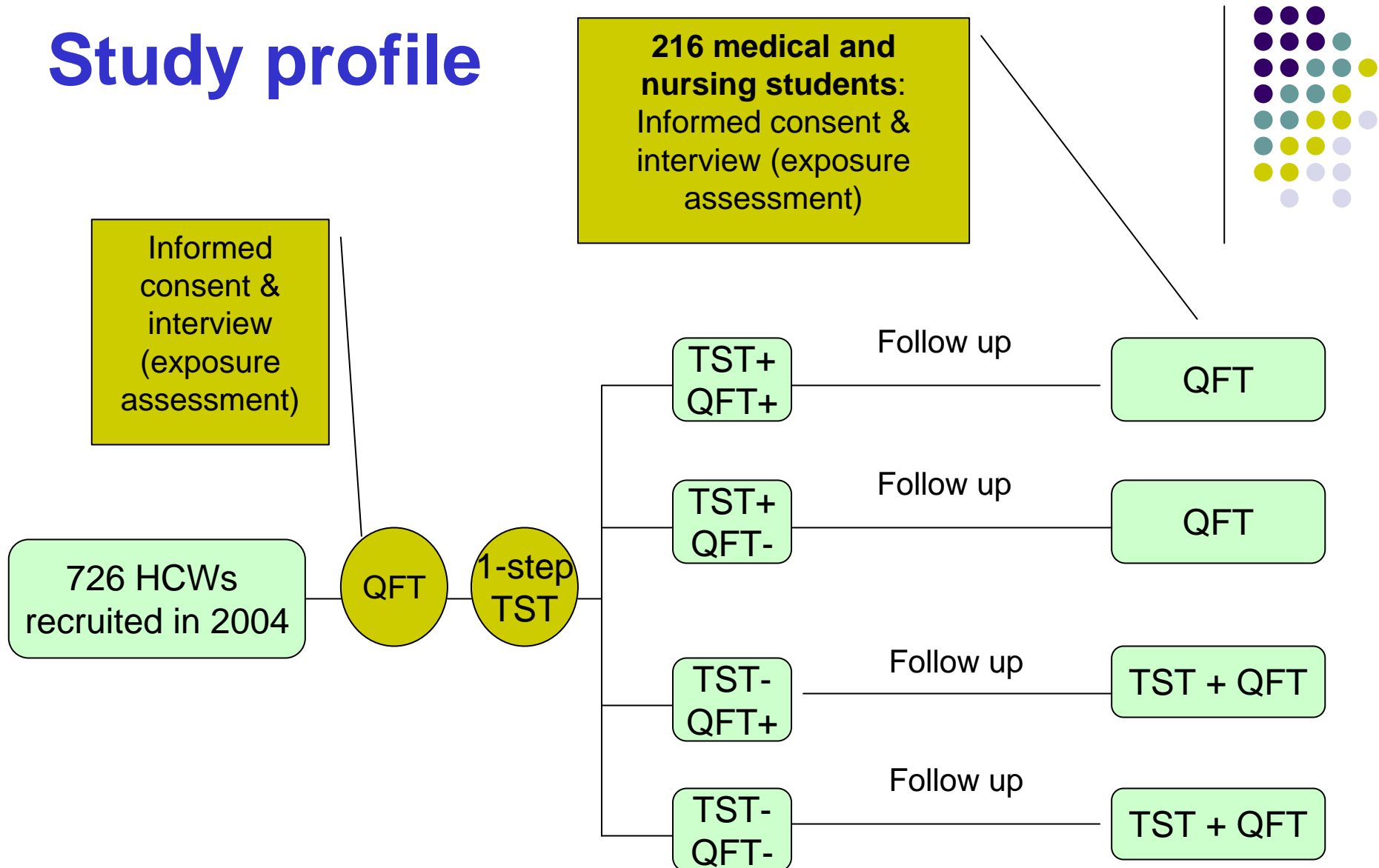
- Future studies should perform careful analyses of correlates of discordance:
 - Not assume that IGRAs are always right
 - Assess if discordance is due to TST and IFN-g values close to their respective cut-points
 - Look for extreme discordance
 - Explore alternative IGRA cut-points and analytic methods:
 - While there is epi evidence to support the TST cut-points, such data do not currently exist for IGRA
 - IGRA thresholds need further validation in various populations
 - Use IGRA results as continuous outcomes
 - Try model based approaches
 - Use serial testing and reproducibility data

IGRA for serial testing in HCWs



- IGRAs are attractive for serial testing because of:
 - Higher specificity
 - No need for 2-step protocol
 - Fewer visits
 - Boosting not a concern
- CDC guidelines: QFT-G can replace TST for serial testing (MMWR 2005)
 - QFT conversion defined as change from neg to pos
- However, to interpret serial IGRA, we need data on:
 - Variability (reproducibility) of IFN- γ responses
 - Test related variability
 - Biologic (within-subject) variability
 - How does TST affect IGRA results?
 - What is an IGRA “conversion”?
 - What is an IGRA “reversion”?

Study profile



18 months between first and repeat testing

Cross-sectional study
 -Completed in 2004
 -JAMA 2005

Repeat TST and QFT
 -In July 2005
 - AJRCCM 2006

Case definitions



- TST “conversion” defined as
 - Initial TST <10 mm, and repeat TST \geq 10 mm, and an increase of 6 mm
 - Initial TST <10 mm, and repeat TST \geq 10 mm, and an increase of 10 mm
- QFT “conversion” defined as
 - Initial IFN- γ <0.35 IU/mL, and repeat IFN- γ \geq 0.35 IU/mL
 - Initial IFN- γ <0.35 IU/mL, and repeat IFN- γ \geq 0.35 IU/mL, and an increase of at least 0.35 IU/mL
 - Initial IFN- γ <0.35 IU/mL, and repeat IFN- γ level twice that of the diagnostic cut-point: IFN- γ \geq 0.70 IU/mL)
- QFT “reversion” defined as
 - Initial IFN- γ \geq 0.35 and follow-up <0.35 IU/mL.

Agreement between QFT & TST at baseline [N=216]



		QFT1 [0.35 IU/mL]			Agreement (%)	Kappa
		QFT+	QFT-			
TST1 [10 mm]	TST+	28	20	48	86	0.57
	TST-	10	158	168		
		38	178	216		

Concordant
positive

Discordant

Concordant
negative

TST conversions in the concordant negative cohort (N=147)



Definition of conversion	N	Conversions	Incidence (95% CI)
≥ 6 mm increment	147	14	9.5% (5.3, 15.5)
≥ 10 mm increment	147	6	4.1% (1.5, 8.7)

QFT conversions in the concordant negative cohort (N=147)



Definition of conversion	N	Conversions	Incidence (95% CI)
IFN- γ \geq 0.35 IU/mL	147	17	11.6% (6.9, 17.9)
IFN- γ \geq 0.70 IU/mL	147	11	7.5% (3.8, 13.0)

Concordance between TST and QFT conversions (less stringent thresholds)



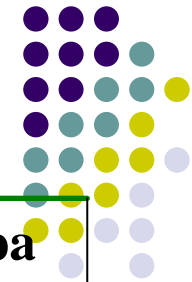
		QFT2 (0.35 IU/mL)			Agreement (%)	Kappa
		+	-			
TST2 [6 mm]	+	9	5	14	91%	0.53
	-	8	125	133		
		17	130	147		

Concordance between TST and QFT conversions (more stringent thresholds)



		QFT2 (0.70 IU/mL)			Agreement (%)	Kappa
		+	-			
TST2 [10 mm]	+	6	0	6	96%	0.70
	-	5	136	141		
		11	130	147		

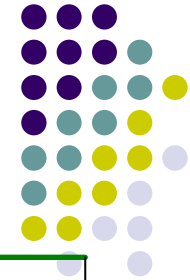
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		QFT2 (0.35)			Agreement (%)	Kappa
		+	-			
TST2 [6 mm]	+	9	5	14	91%	0.53
	-	8	125	133		
		17	130	147		

ID	TST1	TST2	IFNg1	IFNg2
10090	2	5	.14	.35
10238	6	6	0	.62
10203	5	7	.11	.42
10088	2	3	0	.42
10091	1	4	0	.57
10072	2	1	0	.60
10340	6	7	0	.97
10141	1	3	.01	.72

Discordance between TST and QFT conversions (less stringent thresholds)



		QFT2 (0.35)			Agreement (%)	Kappa
		+	-			
TST2 [6 mm]	+	9	5	14	91%	0.53
	-	8	125	133		
		17	130	147		

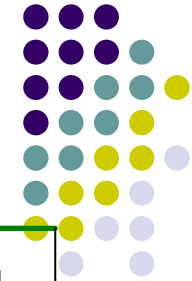
ID	TST1	TST2	IFNg1	IFNg2
10499	3	10	.08	.07
10248	3	10	0	0
10158	5	12	.2	0
10067	5	11	0	0
10192	3	10	0	.24

Concordance between TST and QFT conversions (more stringent thresholds)



		QFT2 (0.70 IU/mL)			Agreement (%)	Kappa
		+	-			
TST2 [10 mm]	+	6	0	6	96%	0.69
	-	5	136	141		
		11	130	147		

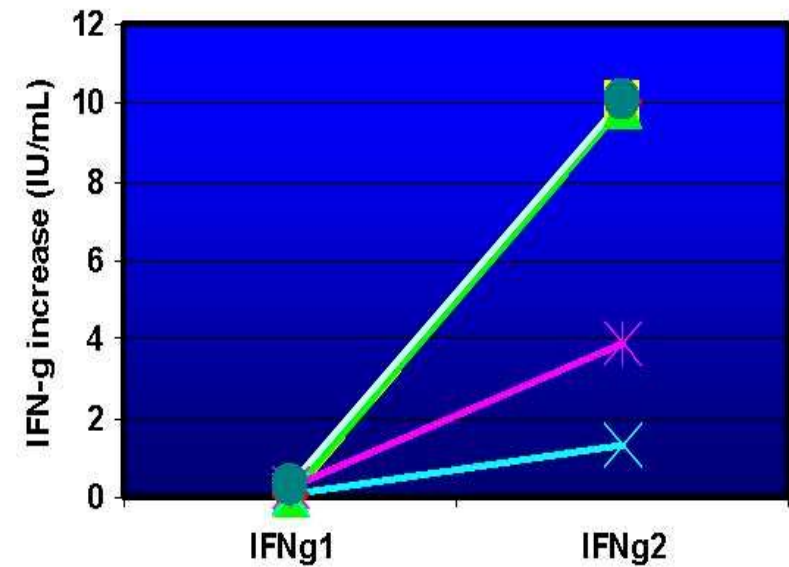
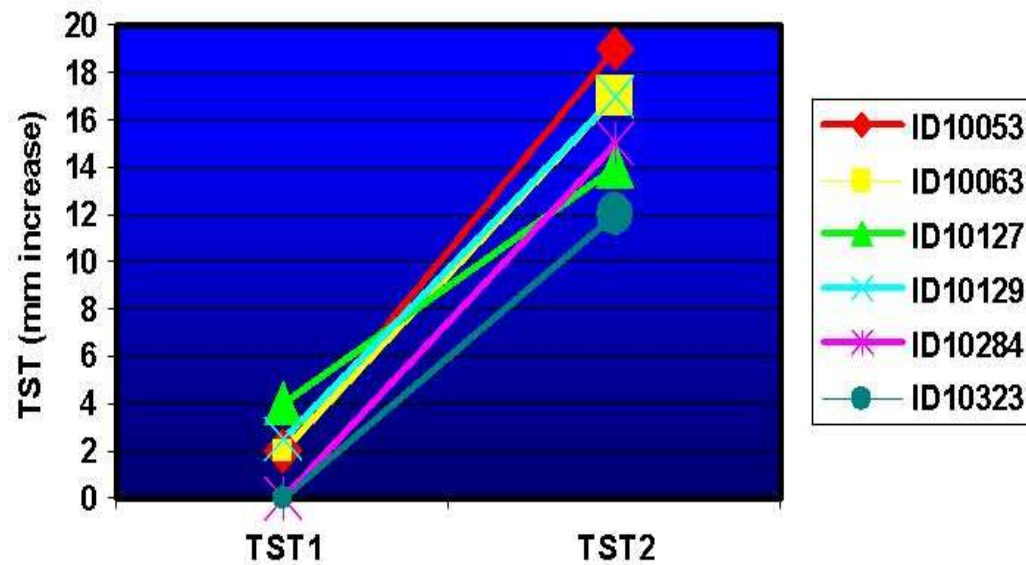
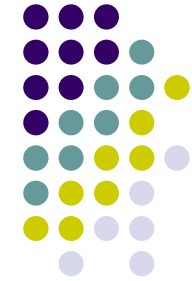
Discordance between TST and QFT conversions (more stringent thresholds)



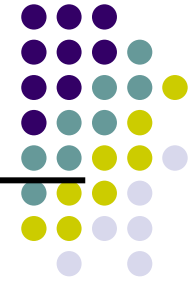
		QFT2 (0.70 IU/mL)			Agreement (%)	Kappa
		+	-			
TST2 conv [10 mm]	+	6	0	6	96%	0.69
	-	5	136	141		
		11	130	147		

ID	TST1	TST2	IFNg1	IFNg2
10080	4	13	0	10
10340	6	7	0	.97
10141	1	3	.01	.72
10263	5	12	0	2.34
10221	1	10	.27	.93

Concordance between TST (10mm) and QFT (0.70 IU/mL) conversions [N=6]

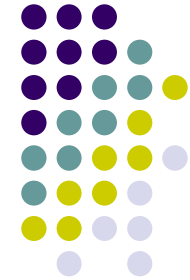


Incidence of TB infection



Definition used	Denominator	Numerator	Incidence (18 months)
TST alone (10 mm)	147	6	4.1%
TST alone (6 mm)	147	14	9.5%
QFT alone (≥ 0.35 IU/mL)	147	17	11.6%
QFT alone (≥ 0.70 IU/mL)	147	11	7.5%
TST 10mm AND QFT ≥ 0.70 IU/mL	147	6	4.1%
TST 10mm OR QFT ≥ 0.70 IU/mL	147	11	7.5%
TST 6mm AND QFT ≥ 0.35 IU/mL	147	9	6.1%
TST 6mm OR QFT ≥ 0.35 IU/mL	147	22	14.9%

QFT reversions



- Baseline TST+/QFT+: 7% [N=28]
- Baseline TST-/QFT+: 70% [N=10]

Baseline IFN-g (IU/mL)	Number re-tested (N)	Total reversions	Incidence of reversions (%)	P-value for trend
0.35 - 0.69	11	6	55	<0.01
0.7 - 1.0	2	1	50	
1.1 – 5.0	9	1	11	
>5.0	16	1	6	
Total	38	9	24	

Discussion



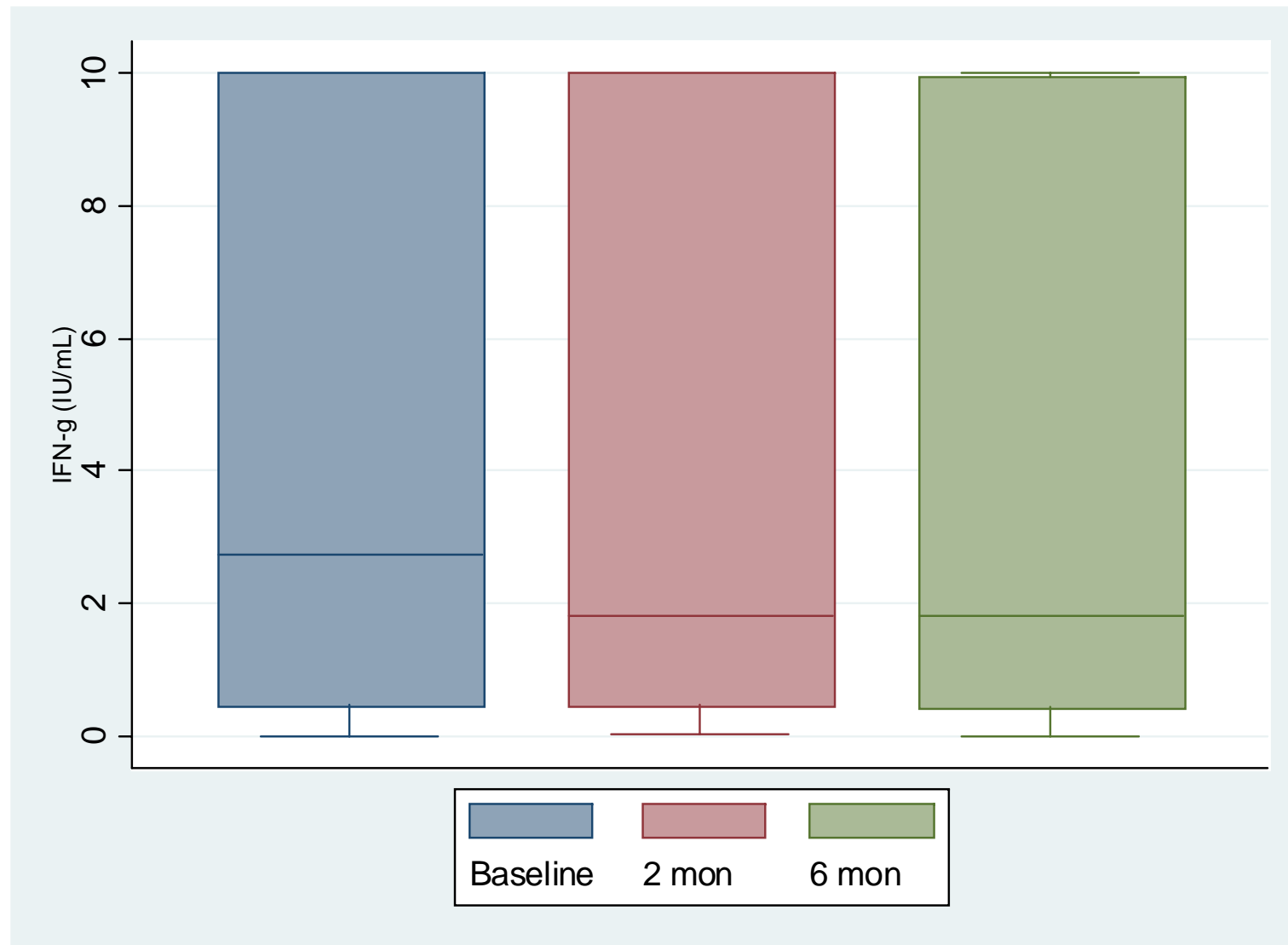
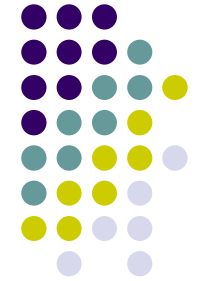
- Conversions, reversions, and non-specific variations occur with serial IGRA
- Thresholds are important
 - different IFN- γ thresholds needed for diagnosis vs. conversion
 - if the diagnostic threshold is used for conversions, non-specific variability might lead to false positive conversions
- QFT-G shows promise for serial testing:
 - strongly concordant with TST conversions at stringent thresholds
 - large TST increases always accompanied by large IFN- γ increases
 - however, to interpret serial IGRA results, we need to determine optimal thresholds to distinguish new infections from non-specific variations

T cell responses after TB therapy



Time	N (smear+ cases)	Sensitivity of QFT-G In Tube	Median IFN- γ (IU/mL)
0 (baseline)	80	74%	2.7
2 mon (intensive phase)	47	81%	1.9
6 mon (treatment completion)	39	79%	1.8

IFN- γ responses at all 3 time points (N=39)



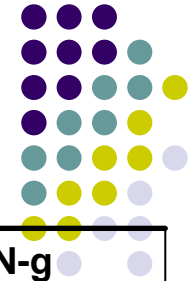
Note: IFN-g levels >10 IU/mL have been analyzed as 10 IU/mL

Discussion

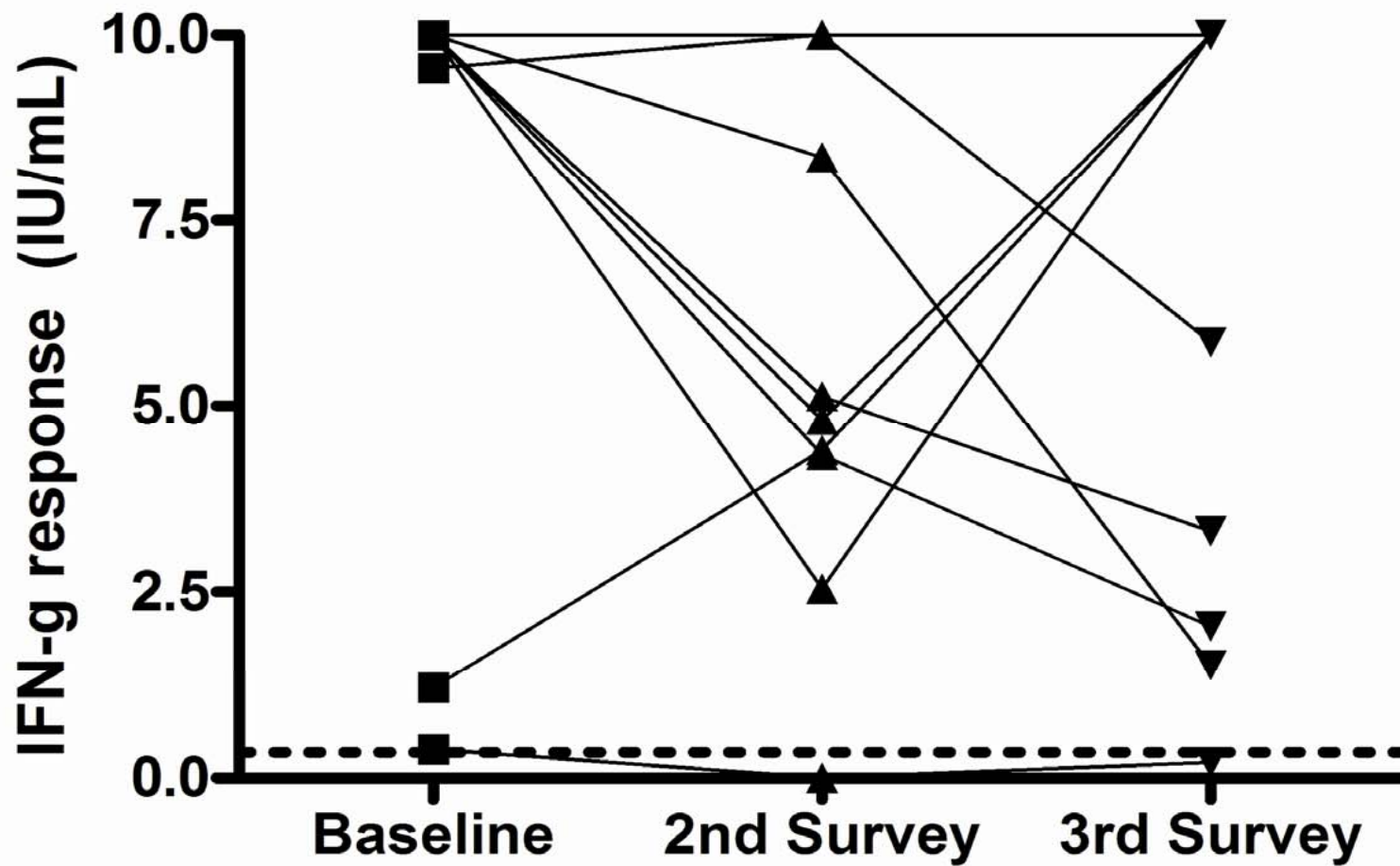


- Among TB patients with mostly moderate to advanced disease, QFT-G had a sensitivity of 74%
- No significant change in sensitivity during the course of treatment
- When QFT results were analyzed as dichotomous measures, results were largely unchanged during the course of treatment
- When QFT results were analyzed as continuous measures, inconsistent changes were seen mostly at levels higher than the cut-point of 0.35 IU/mL

T cell responses after preventive therapy: 10 HCWs after 6 months of INH



ID	Age	Sex	BCG	TST	IFN-g at baseline (Jan/Feb 2004)		IFN-g 4 mon after Rx (Jan 2005)		IFN-g 10 mon after Rx (July 2005)	
					Result	IU/mL	Result	IU/mL	Result	IU/mL
10055	19	F	Y	14	P	10.0	P	10.0	P	10.0
10066	19	F	Y	15	P	10.0	P	2.55	P	10.0
10069	19	F	Y	17	P	9.55	P	10.0	P	10.0
10077	19	F	N	19	P	10.0	P	4.34	P	2.04
10082	19	F	Y	18	P	10.0	P	10.0	P	5.86
10089	19	F	Y	16	P	10.0	P	8.35	P	1.51
10093	19	F	N	20	P	10.0	P	5.13	P	3.31
10115	24	F	Y	17	P	0.39	N	0	N	0.21
10282	19	F	N	15	P	1.21	P	4.40	P	10.0
10290	18	F	N	18	P	10.0	P	4.81	P	10.0
Mean						8.1		5.9		6.3
Median						10.0		5.0		7.9



Note: IFN-g levels >10 IU/mL have been analyzed as 10 IU/mL; broken line indicates QFT cut-point of 0.35 IU/mL

Discussion



- In a setting with ongoing, intensive exposure, HCWs have high IFN-g responses at baseline, and continue to have persistently elevated responses, despite LTBI treatment
- Due to:
 - **Persistence** of the original infection?
 - 6 months INH not adequate?
 - INH resistance?
 - Need to wait longer for T cell responses to go down?
 - Due to exogenous **re-infection**?
 - Raises concerns about efficacy of preventive therapy in high incidence settings with recurrent exposure
 - Are those with persistently elevated T cell responses more or less likely to progress to active disease??

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- Rick O'Brien, FIND

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