

Interpretation of IGRA Results – A View from the Clinical Lab



Aker-Zornkovic Photography

Methodist The Methodist Hospital
Research Institute

Edward A. Graviss, PhD, MPH
eagraviss@tmhs.org

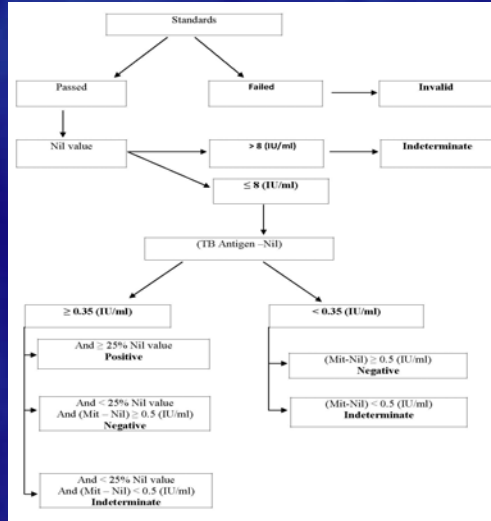
TMH Clinical Lab

- CLIA certified 2006 (BCM) - QFT
2008 (TMH) - QFT and T-SPOT
- Client(s) make choice of which IGRA assay to request
 - Training provided (in-service and/or shadowing)
- Free consultation provided when requested, based on:
 - Risk of pool population
 - Logistics of blood draw
 - Previous IGRA results
 - Timing of TST (3 – 28 days)
 - Prevalent literature (including meta-analyses)

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QuantiFERON Interpretation Flow Diagram



QuantiFERON Client Breakdown

- Total Clinical Tests done since Sept '06 - 11560
 - ExxonMobil – 7073
 - Hospitals / Medical Center – 1983
 - Private Doctors / Other – 711
 - Chevron - 1766
 - NASA Clinical Testing – 27
- NASA Study – 100
- CDC Task Order #18 – 2631

QuantiFERON Quality Control

- QFT-IT is repeated on the same specimen if:
 - Invalid due to any failed standard
 - All indeterminate – low mitogen (most cases) or nil > 8 IU/ml
 - Negative result with high nil
 - Negative result when the (TB Ag – nil) value = 0.25 to 0.35
 - All positive results

** (If two results are discordant, the sample is duplicated the next day; if results cannot be determined after 4 assays, a re-draw in 1 month is recommended)

Overall QuantiFERON Clinical Results

- Positive Results – 902 – 7.80%
- Negative Results – 10198 – 88.23%
- Indeterminate Results – 453
 - Due to high Nil - 62
 - Due to low Mitogen – 389
 - Other Conditions – 2
- Indeterminate Rate – 3.92% [453/11560]

2.2%: J Rothel
Unpublished data
2008 (n = 36)

Special QuantiFERON Results – Borderline

- The cutoff-point for positive readings is:
Antigen – Nil = 0.35 IU/ml
- Borderline assays have Antigen – Nil readings
between 0.25 and 0.45 IU/ml
- Borderline Assays – 245 (2.12%, 245/11560)
 - Borderline Positive – 103 (11.42%, 103/902)
 - Borderline Negative – 142 (1.39%, 142/10198)

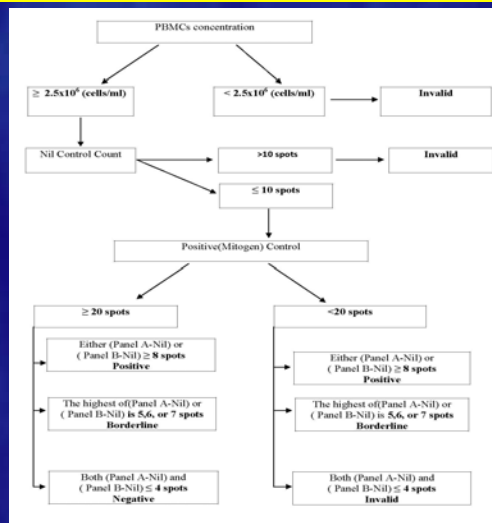
QuantiFERON Repeat Testing (since 7/2009)

- Total tests = 7520
- Specimens repeated = 1194 (15.9%)
 - Positives = 516
 - Negatives = 356
 - Indeterminates = 322
- Single repeats – 1085 (90.9%)
 - Second repeat - 47
 - Triple repeat - 57
 - Quad repeat – 5
- Flip Flop results – 62 (>2 repeats)
 - 5.19% of repeated testing
 - 0.82% of total tests since 7/2009

T-SPOT.TB Assay Procedures

- Lithium or sodium heparin tubes preferred
- Specimen processing within 8 hours of phlebotomization in a biosafety cabinet (BSC)
 - T-Cell Xtend® reagent allows extended processing delay of 23-30 hours post venipuncture
 - Volume of blood accepted: 1 – 10 ml
 - Ficoll separation method used for PBMC isolation (alt: Leucosep™)
- Mindray (BC-3200) cell counter
 - PBMC hand-counting can be done with hemocytometer
- Spot-counting via stereoscope (2-3 independent readers)
 - CTL Immunospot Analyzer is available for spot clarification, research studies and pictures

T-SPOT Interpretation Flow Diagram



T-SPOT Client Breakdown

- Total Clinical Tests done since Aug '08 - 1339
 - ExxonMobil / Chevron – 50
 - Hospitals / Medical Center – 150
 - The Methodist Hospital and clinics – 838
 - TMH Employee Health – 169
 - Others (clinics) – 14
 - Kidney Transplant Groups – 60
 - Refugees – 58

T-SPOT Results

	Positive	Negative	Borderline	Indeterminate	Invalid / Failed	Total
Jail Study ¹	74	316	0	22	0	412
IDU Study ²	41	82	0	0	0	123
BTGH	50	51	0	9	0	110
UTP ³	38	56	5	0	5	104
TO18 [^]	243	2079	134	0	173	2629
Clinical	209	1036	64	0	30	1339

¹ Porsa E et al. Clin Vaccine Immunol 2007; 14:714-719

² Grimes CZ et al. Int J of Tuberc Lung Dis 2007; 11:1183-1187.

³ Cruz AT et al. Pediatrics 2011; 127; e31-8.

[^] T-SPOT not done on 2 sample; use of 4 ml CPT tubes

The Unexpected T-SPOT Results

(Unexpected results include indeterminate and failed)

Result	Cause(s)	Number of cases	Solution
Indeterminate / Failed**	The Nil Control has more than 10 spots	50/4717 (1.06%)	Re-sample one month later
	The Positive Control has less than 20 spots	36/4717 (0.76%)	
Failed	The cell number added in each well for detection is less than 250,000***	136/4717 (2.88%)	Re-sample as soon as possible and take an extra tube
	Other – Spot clumping, contamination, etc.	17/4717 (0.36%)	

** Indeterminate and Failed results were merged during the UTP study. Results have been categorized as they were initially reported.

*** If the result would be Positive with a valid cell count, it will be reported as Positive

Graviss Lab Statistics

- QFT Overall (+) Rate – 7.80% (902/11560)
- QFT Overall (-) Rate – 88.23% (10198/11560)
- QFT Indeterminate Rate – 3.92% (453/11560)

- T-Spot Overall (+) Rate – 13.89% (655/4717)
- T-Spot Overall (-) Rate – 76.74% (3620/4717)
- T-Spot Borderline Rate – 4.30% (203/4717)
- T-Spot Fail/Indeterminate Rate¹ – 5.07% (239/4717)

¹Fail/Indeterminate rate drops to 2.59% (119/4597) when TO18 low cell counts are excluded (5 ml CPT tubes)

Case Presentation (1)

- Forty-six year old WM, German National, working for a large petrochemical company as a chemist. Full physical done in 11/2011 as part of his relocation to Canada.
- Company OHP switched from TST to QuantiFERON ≈2 years ago. Physical was unremarkable other than a positive QuantiFERON (CXR-, BCG-).
- As part of the screening algorithm the scientist was identified as having LTBI and was to begin 6 months of INH therapy.
- The worker tested TST- in California in 11/2008, but spent 8 months in 2010 with family in Manama, Bahrain working in a corporate lab. TB incidence in Bahrain = 2/100,000.
- Worker questioned OHP nurse about variability of QFT and results. Wanted second opinion from a second clinician and lab.

Case Presentation (2)

- 11/8/2011 Initial QFT = 56 IU/ml. Large reference lab in Houston
- 11/15/2011 second QFT drawn at TMH. T-SPOT. *TB* was also drawn at the same time.
- Second QFT: nil = 0.14 IU/ml; antigen 0.13; mitogen = >10 IU/ml
- T-SPOT. *TB*: nil = 0 SPU; ESAT-6 = 0 SPU; CRP-10 = 0 SPU; mitogen = TMTC
- Follow up with OHP nurse
 - “Initial lab never reports out quantitative results”. No storage capacity specimens thrown out after 2 days
 - “increased QFT+ results over the last several months”
 - Change in screening algorithm by petrochemical OHP

Discussion

- Inherent variability for all laboratory assays. For immunologic assays variability associated with both the subject and the laboratory.
- Minimize laboratory variability by conservative approach and increased quality control, including retesting of the same clinical samples multiple times.
- Utilize all the available tools in your tool box to define whether the patient is LTBI or not, because the downstream ramifications are significant, not only public health but personal as well.
- Communication between lab and provider essential

Questions ?

TMH IGRA Lab



Justin Lew , BS



Ngan Ha , BS



Sophie Im , BS