

QUANTIFERON-TB GOLD IN-TUBE OD READINGS AROUND THE CUT-OFF VALUE: WHAT DO THEY MEAN?

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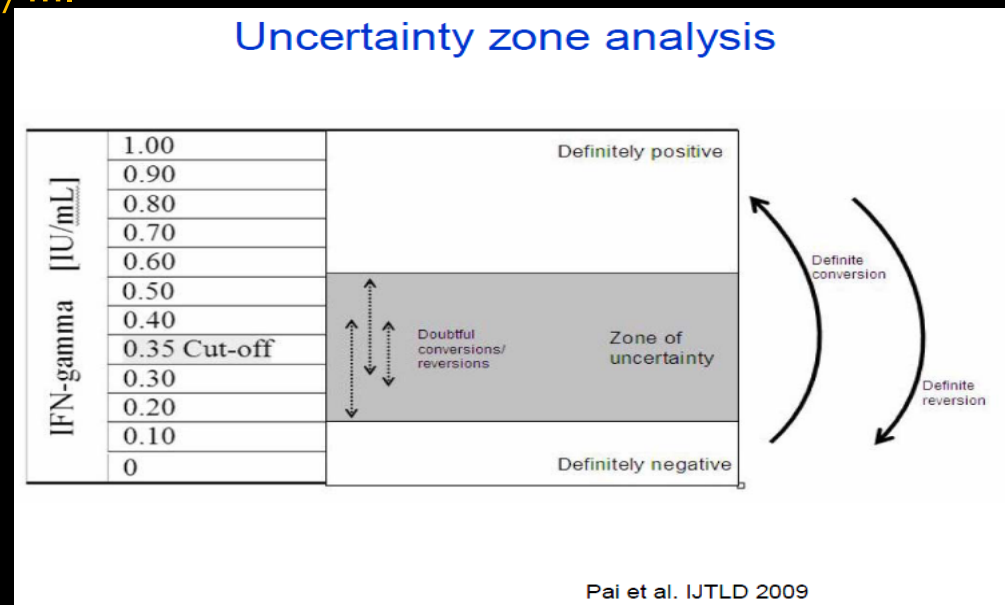
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Uncertainty zone:

- The QuantiFERON-TB Gold In-Tube assay (based on ELISA detection of INF γ response to *M.tb*) has a **threshold of 0.35IU/ml.**
- IGRA conversions and reversions are not well understood!
- Interpretation needs more evidence: (Pai M; O'Brien,R. PLoS Med 2007 4(6)e208).; Hill P et al PLoS Med 2007 4:e192
 - Reproducibility of T-cell response over time (within-subject variations)
 - Threshold definitions required for progression to disease?
 - Test-related error/non-specificity

Uncertainty zone:

- Positive values vary more than negative values (range vs commercial cut-off).
- Harada N et al 2004 Kekkaku 79:725-35 Japanese
 - 'grey zone' 0.1-0.35IU/ml
- Pai, M et al Int J Tuberc Lung Dis 2008 13(1):84-92
 - Arbitrarily chose 0.2-0.5IU/ml

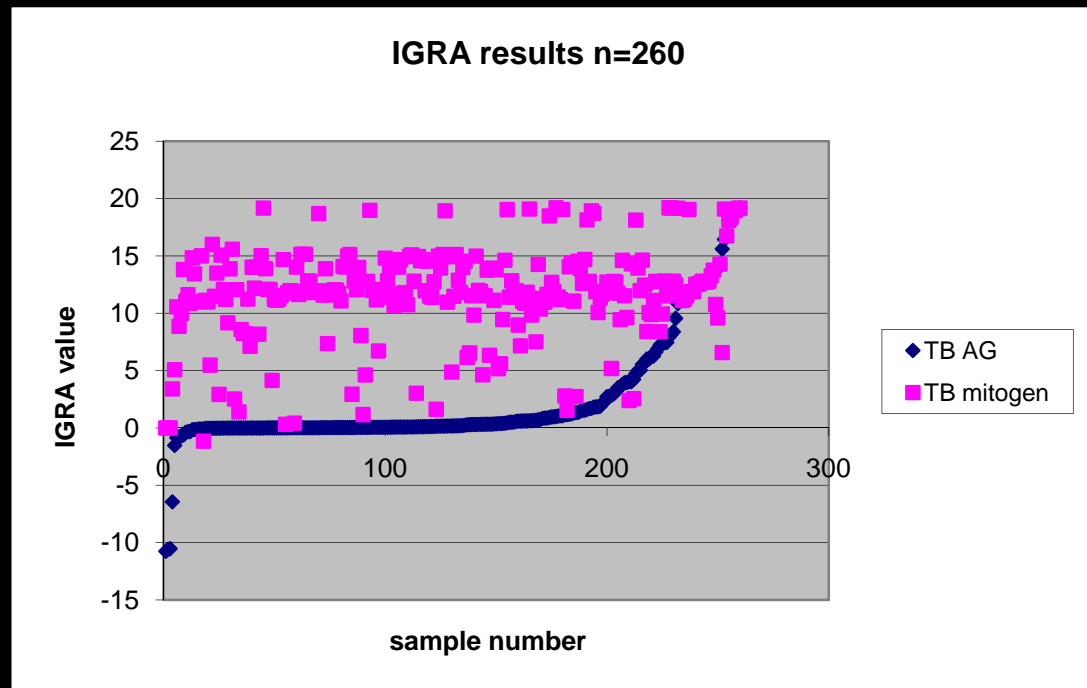


How much variation is due to laboratory tests?

- Longitudinal cohorts being followed in a high endemic TB area, Johannesburg, South Africa:
 - Medical students (n=79 enrolled) entering their first predominantly clinical year of study (3rd) : TST vs QuantiFERON Gold In-Tube Assay.
 - HCW (n=118) in a TB and HIV ARV treatment clinic TST vs QuantiFERON Gold In-Tube Assay.
- Laboratory investigation:
 - Accuracy: n=27 analysed in duplicate
 - Precision: n=2 diluted 1:10, 1:20, 1:40 in negative plasma and repeated 6 times (Nil and TBAg). Single ELISA plate.
- Bland-Altman and SD reported.

Data range: n=260

- Total samples analysed over both cohorts:
 - ▣ TB antigen: Range:-10.76 to 19.16IU/ml;
Median=0.17IU/ml.
 - ▣ Mitogen: Range: -1.16 to 19.19IU/ml; median
11.94IU/ml.



Accuracy at 0.35IU/ml:

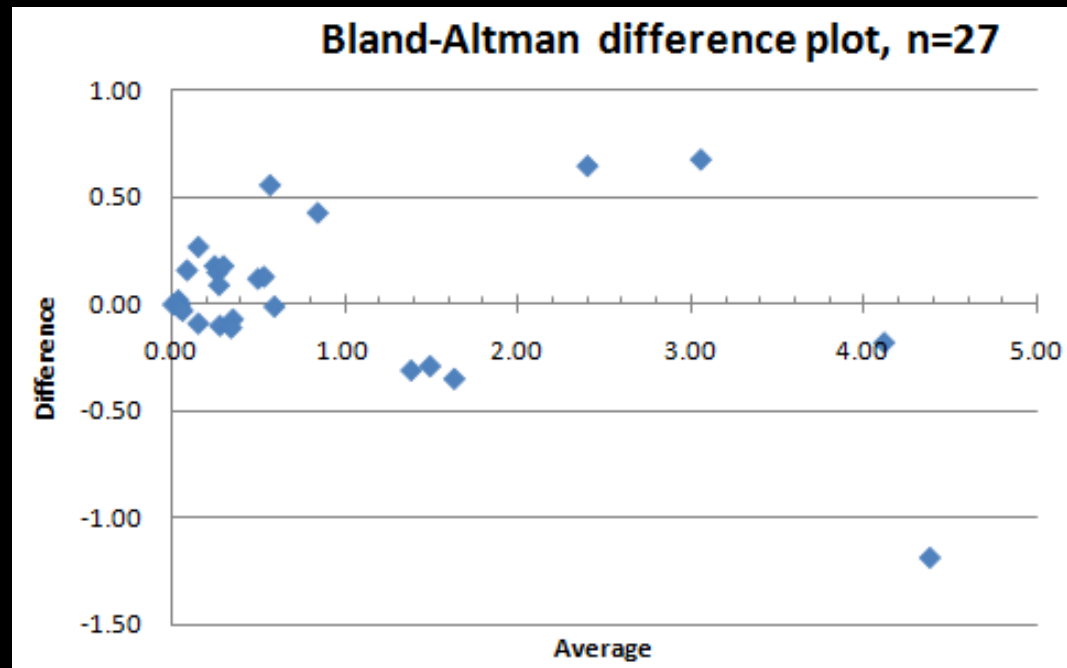
□ N=27 samples analysed in duplicate:

□ Range: 0.01 to 4.98IU/ml. Mean 0.88IU/ml

□ Mean difference: 0.03IU/ml

□ 4 /27 (14.8%) discordant at 0.35IU/ml

1	2			average	Difference
0.01	0.01	N	N	0.01	0.00
0.01	0.17	N	N	0.09	0.16
0.02	0.29	N	N	0.16	0.27
0.03	0.05	N	N	0.04	0.02
0.05	0.05	N	N	0.05	0.00
0.06	0.04	N	N	0.05	-0.02
0.08	0.05	N	N	0.07	-0.03
0.16	0.34	N	N	0.25	0.18
0.19	0.34	N	N	0.27	0.15
0.2	0.11	N	N	0.16	-0.09
0.21	0.39	N	P	0.30	0.18
0.23	0.32	N	N	0.28	0.09
0.29	0.85	N	P	0.57	0.56
0.33	0.23	N	N	0.28	-0.10
0.39	0.32	P	N	0.36	-0.07
0.4	0.29	P	N	0.35	-0.11
0.44	0.56	P	P	0.50	0.12
0.47	0.6	P	P	0.54	0.13
0.6	0.59	P	P	0.60	-0.01
0.63	1.06	P	P	0.85	0.43
1.54	1.23	P	P	1.39	-0.31
1.64	1.35	P	P	1.50	-0.29
1.81	1.46	P	P	1.64	-0.35
2.08	2.73	P	P	2.41	0.65
2.72	3.4	P	P	3.06	0.68
4.21	4.03	P	P	4.12	-0.18
4.98	3.79	P	P	4.39	-1.19



Precision at 0.35IU/ml

n=2 1:10, 1:20, 1:40

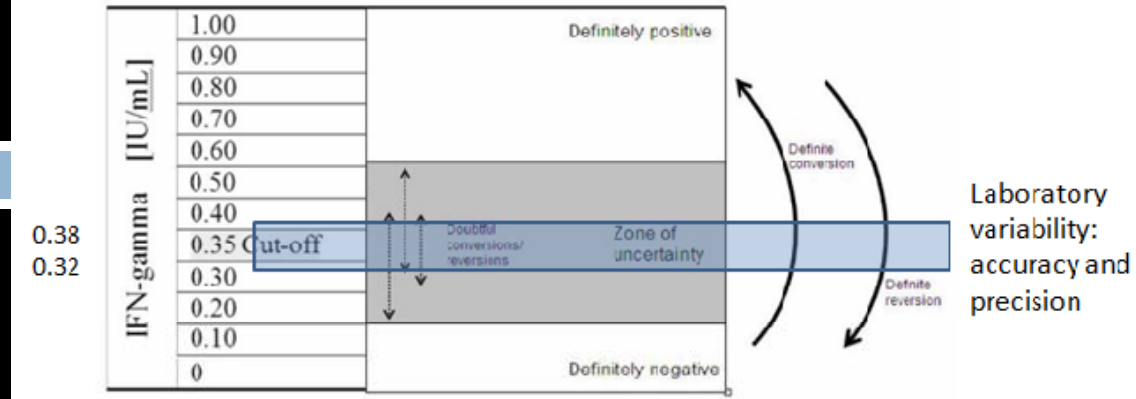
- Range in values: 0.1 to 0.63IU/ml
- SD of precision: 0.02IU/ml (range 0.01 to 0.05IU/ml)
- CV: 2.9% at 1:10 to 14.6% at 1:40

s97	repeats					
10	0.54	0.57	0.57	0.53	0.55	0.56
20	0.31	0.26	0.28	0.28	0.26	0.25
40	0.14	0.11	0.1	0.1	0.12	
s112						
10	0.63	0.58	0.59	0.56	0.61	0.69
20	0.27	0.27		0.25	0.28	
40	0.15	0.15	0.15	0.15	0.13	

Conclusion

- Accuracy:
 - ▣ **Quantitative differences:** Repeat testing on the same specimen (accuracy) showed small differences (0.03IU/ml) with a positive bias.
 - ▣ **Qualitative differences:** 85% samples showed concordance around the 0.35IU/ml cut-off . 96.4% previously described (Veerapathran A et al PLoS 2008:3(3)e1850)
- Precision: at 0.35IU/ml found to be 0.02IU/ml

Conclusions



- Optimal thresholds need to be defined:
- Our findings: taking accuracy and precision into account: a value of **0.35IU/ml** could represent any value between **0.32 and 0.38**, due to laboratory/assay variability.
- Suggest:
 - Values between **0.32 - 0.34** should **not** be interpreted as **negative**
 - Values between **0.35 - 0.38** should **not** be interpreted as **positive**
 - Intra-individual changes over time in this 'intermediate' range should **not** be interpreted as QTF conversions or reversions.
 - **Report both qualitative and quantitative** values for interpretation.

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