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Short Communication

TB Sure®: Its Place in Diagnosis of Tuberculosis

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Abstract

Cellular immune response play important role in control of infection with both CD 4+ helper T cells and CD 8+ Cytotoxic T cells playing important roles against *Mycobacterium tuberculosis* (*M tb*) in granuloma formation. While CD 4+ cells play to activate macrophages and prevent development of tuberculosis, recently it has been determined that CD 8+ cytotoxic cells play an important role in decreasing bacterial load and better disease control.

Recent evidence indicate that detection of CD 8 + T cell response can differentiate between active tuberculosis and LTBI. The sensitivity of immunological based assays in discriminating between active and LTBI is inferior to molecular methods such as Gene xpert or liquid culture, but the advantage of fourth generation Quantiferon TB Gold plus, which we have labelled as TB Sure® test is that when the location of disease cannot be determined or when relevant sample can not be collected and submitted for culture and Gene xpert analysis. The test is performed on peripheral blood collected in lithium heparin vial.

Since TB specific CD 8+ cytotoxic represents multiplication of *M tb* within macroploye, TB Sure® test, can help identify bacterial multiplication and its subsidence on adequate treatment when a patient is placed on effective anti TB treatment when the test is performed at 0, 3 and 6 months TB Sure® is also an appropriate test for contact tracing, being more specific than Tuberculin Skin Test of Quantiferon TB Gold or Platinum test.

Keywords: TB Sure®; Tuberculosis

Introduction

Worldwide, Tuberculosis (TB) is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS). Millions of people continue to fall sick with TB each year. In 2017, TB caused an estimated 1.3 million deaths (range, 1.2 - 1.4 million) among HIV-negative people and there were an additional 300 000 deaths from TB (range, 266,000 – 335,000) among HIV-positive people. Ten million people (range, 9.0 - 11.1 million) developed TB disease in 2017: 5.8 million men, 3.2 million women and 1.0 million children. There were cases in all countries and age groups, but overall 90% were adults (aged ≥15 years), 9% were people living with HIV (72% in Africa) and two thirds were in eight countries: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and South Africa (3%). These and 22 other countries in WHO's list of 30 high TB burden countries accounted for 87% of the world's cases. Drug-resistant TB continues to be a public health crisis. The best estimate is that, worldwide in 2017, 558 000 people (range, 483 000-639 000) developed TB that was resistant to rifampicin (RR-TB), the most effective first line drug, and of these, 82% had multidrug-resistant TB (MDR-TB). Three countries accounted for almost half of the world's cases of MDR/RR-TB: India (24%), China (13%) and the Russian Federation (10%). Worldwide, the TB incidence rate is falling at about 2% per year. Effective utilization of the available diagnostic methods followed by individualised treatment can help decrease this rate to 10%. In addition, about 1.7 billion people, 23% of the world's population, are estimated to have a latent TB infection, and are thus at risk of developing active TB disease during their lifetime [1].

Natural history of tuberculosis

Mycobacterium tuberculosis is an obligate pathogen normally acquired through respiratory tract (Figure 1). After infection, establishment of human response is able to contain microbial growth in 90 - 95% cases; this process is characterized by activation, recruitment and/or proliferation of distinct cells in the infectious foci, growth control and confinement of the pathogen inside a granuloma, a condition called as latent TB. In contrast, immune compromised-associated conditions lead to active disease, characterized by microbial growth, tissue damage and antibody production. Case finding of active cases is critical to stop TB transmission. Antibodies, depicted using the symbol Y are neither of diagnostic importance nor protective in nature, but antigens liberated from bacteria may serve as biomarkers of this infection stage [2].

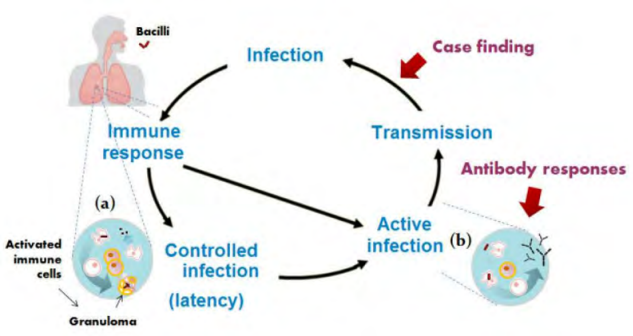


Figure 1: Natural history of tuberculosis [2].

Adaptive responses take place when lymphocytes recognize mycobacterial molecules as foreign entities (as antigens) (Figure 2). Two lymphocyte families, the T and B-cells, are activated after encountering with their matching antigen, then develop into the effectors of adaptive immunity. For T cells, presentation of foreign entities by antigen-presenting cells is mandatory for recognition. In contrast, B cells recognize their cognate antigens through direct interaction via the B-cell receptor. Once activated, B and T cells trigger a variety of functions, mainly including: (a) secretion of cytokines and chemo-attractants by CD4+ helper T-cells, (b) lysis of infected cells via the release of lytic enzymes by CD8+ cytotoxic T-cells, (c) secretion of antibodies by plasma B-cells that have been derived from activated B lymphocytes, (d) production of a number of long-lived memory T- and B-cells, which last for many years circulating in the bloodstream and monitor for infection (either newly developed or endogenously re-activated).

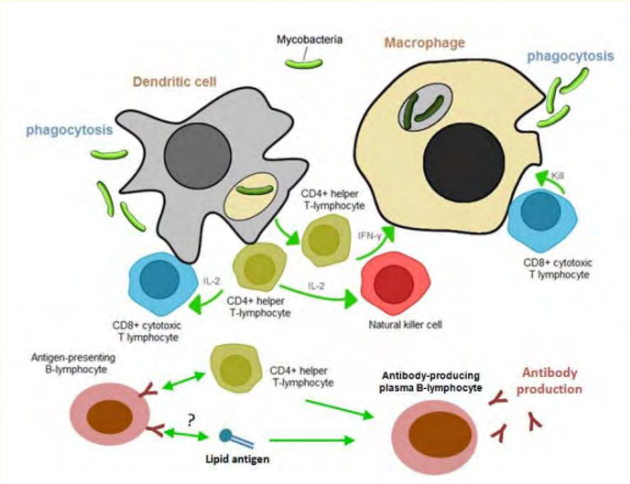


Figure 2: Adaptive immune response in Tuberculosis [2].

Immune responses related to cell-mediated immunity are restricted to active infection. One illustrative example is the release of some pro-inflammatory cytokines by T-cells, such as interferon- γ. These immune responses during active infection are known to be critical for arresting the growth of *M. tuberculosis* [2].

Cell Mediated Immunity Based Diagnosis Of TB

Despite intensive investigations, rapid formulation of diagnosis of active tuberculosis remains a Rozot., *et al*. [3] reported that by using flow cytometry the cytokine profile of *Mycobacterium tuberculosis* (Mtb)-specific CD4 T cells allowed a strong immunological discrimination between patients with active tuberculosis and latent Mtb infection (LTBI). They subsequently confirmed that Mtb-specific CD8 T-cell responses were predominantly (> 70%) found in patients with active tuberculosis compared to those with LTBI (15%). On the basis of these previous observations, they hypothesized that the combined assessment of Mtb-specific CD4 and CD8 T-cell responses could result in improved diagnosis of active tuberculosis. They analyzed both the functional profile of Mtb-specific CD4 T-cell responses and the presence of Mtb-specific CD8 T-cell responses in 194 subjects diagnosed with active tuberculosis or LTBI, and performed multivariate regression analysis to assess their relative or combined capacity to distinguish active tuberculosis from latent infection. The results showed that both individual immunological measures had variable power to discriminate between active tuberculosis and LTBI. However, the combination of both measures

greatly improved the power of this flow cytometry-based assay in the diagnosis of active tuberculosis [4].

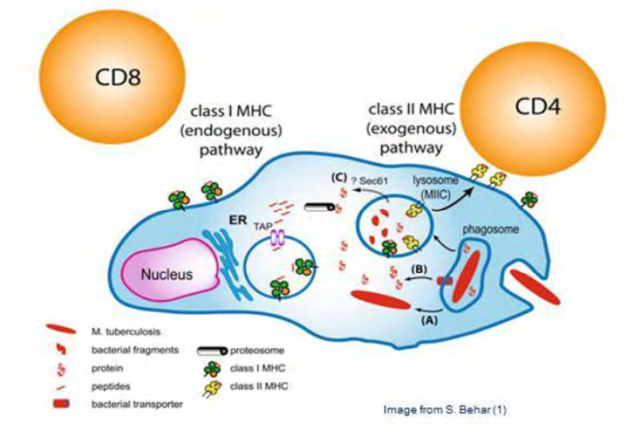


Figure 3: The central role of macrophages in controlling Tuberculosis and actions on infected macrophages by activated CD 4+ and CD 8+ T lymphocytes via antigen presenting cells.

The current model for the protective role of CD8+ T cells in TB is based on the ability to lyse infected macrophage cells (in addition to cytokines release), On investigating whether the bacterial load (i.e. smear positive versus smear-negative TB) or the clinical presentation of TB disease (i.e. PTB versus ETB) correlated with distinct profiles of *Mtb*-specific CD8+ T-cell responses. A higher prevalence of *Mtb*-specific CD8+ T-cell responses in PTB compared with ETB and a higher magnitude of these responses in smear-positive versus smear-negative PTB patients was observed. There was limited proliferation capacity of *Mtb*-specific CD8+ T cells from PTB patients compared with ETB patients. This is consistent with the current paradigm associating CD8+ T-cell responses to high antigen burden. Furthermore, as mentioned above, these functional differences may reflect different conditions in the stimulation of the immune responses in the different anatomic sites, to the tropism of responding T cells or to distinct stages of disease. Overall, several phenotypic and functional differences in the profiles of *Mtb*-specific CD8+ T cells between patients with active disease and LTBI subjects were identified [5].

Notwithstanding the key role of CD4+ T cells in the control of *Mtb* infection, there was a strong association between profiles of *Mtb*-specific CD8+ T cells and distinct clinical presentations. In particular, significant differences in the phenotypic (e.g. T-cell differentiation) and functional (e.g. GrmA expression) profiles between patients with active TB disease and subjects with latent infection was identified. Whether these phenotypic and functional profiles reflect different levels of immune control remains to be determined. Recent studies also questioned whether LTBI subjects represent a model of efficient control of *Mtb*. In this regard, there is growing evidence that LTBI corresponds to a broad spectrum of subjects with *Mtb* infection ranging from exposed uninfected subjects to subjects with subclinical TB. One could speculate that the presence of CD8+ T cells (found in 15% of LTBI) may represent a marker of truly chronically infected subjects and potentially identify subjects at risk of reactivation (occurring in about 10% of subjects) that would benefit from chemoprophylaxis. Longitudinal studies are needed to confirm this hypothesis [6].

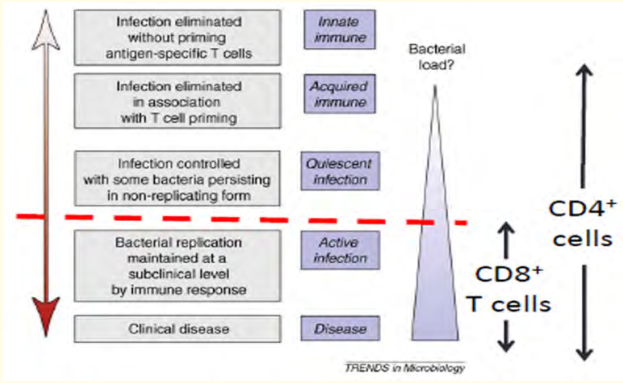


Figure 4: The range of activity of specific CD 4+ and CD 8+ cells in containing Tuberculosis.

Fourth generation igra

The QuantiFERON-TB Gold Plus (QFT-Plus) (Pathkind will use TB SURE® as a trade name to avoid confusion with Quantiferon Gold Test) represents the new QuantiFERON-TB Gold In-tube (QFT-GIT) to identify latent tuberculosis infection (LTBI) as well as detect recent expose and active infection.. The main differences is the addition of a new tube containing shorter peptides stimulating CD8 T-cells. In order evaluate the accuracy of TB Sure® compared with QFT-GIT in a cross sectional study of individuals with or without tuberculosis (TB). Blood sample in lithium heparin vial were collected from 179 participants: 19 healthy donors, 58 LTBI, 33 cured TB and 69 active TB. TB Sure® and QFT-GIT were performed. The two tests showed a substantial agreement. A similar sensitivity in active TB and same specificity in healthy donors. A higher proportion

of the LTBI subjects responded to both TB1 and TB2 compared to those with active TB (97% vs 81%). Moreover, a selective response to TB2 was associated with active TB (9%) and with a severe TB disease, suggesting that TB2 stimulation induces a CD8 T-cell response in absence of a CD4-response. Interestingly, a higher proportion of the LTBI subjects responded concomitantly to TB1 and TB2 compared to those with active TB, whereas a selective TB2 response associated with active TB.

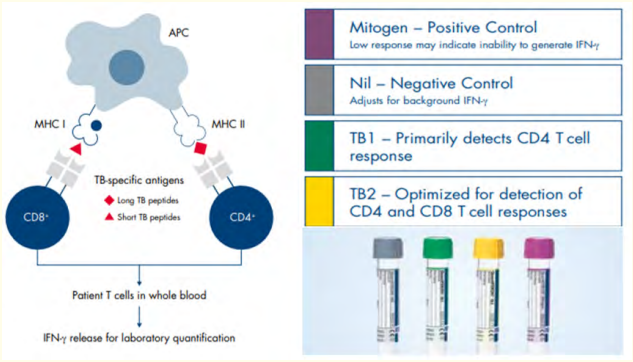


Figure 5: The four tubes used in The Quantiferon Gold TB Plus (TB Sure®) test.

According to the manufacturer, TB1 peptides have been designed to stimulate CD4+ T cells while TB2 should elicit both CD4+ and CD8+ responses. Recently, studies using QFT-Plus kit (TB Sure®) showed that the difference in IFN-ϒ production between TB2 and TB1 stimulation may provide a surrogate marker of the CD8+ T cell response magnitude. This difference has been associated with smear positivity in active TB patients or with a recent exposure in TB contacts.

To perform TB Sure® test atleast 4 ml of whole blood in lithium heparin tube is required. One ml each is placed into each of the four tubes and incubated at 37 C for 16 to 24 hours followed by quantitative determination of Interferon γ by ELISA.

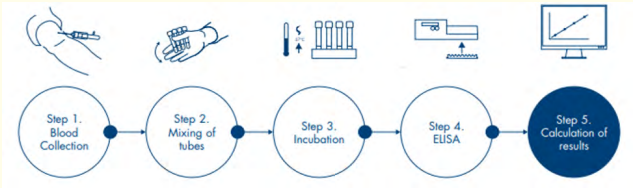


Figure 6: Workflow of the TB Sure® test.

In the QFT-Plus test (TB Sure®), a significant difference in interferon-γ response between the two tubes would indicate recent infection or active infection. A previous study analysed the contribution of CD4+ and CD8+ antigen-specific responses to novel peptides with promiscuous human leukocyte antigen II binding capabilities derived from *M. tuberculosis* genes over-expressed in an *in vitro* macrophage model a small subgroup of active tuberculosis patients had a significantly higher frequency of peptide-specific interferon-γ CD69+ CD4+ and interferon-γ CD69+ CD8+ T-cells compared to controls.

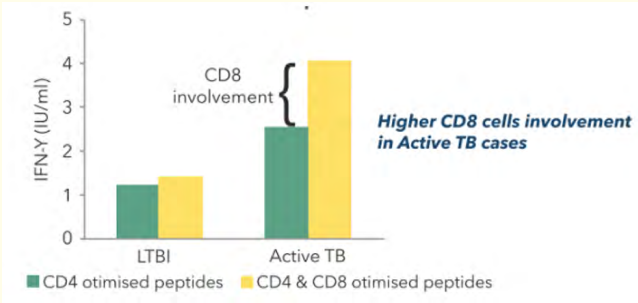


Figure 7: The difference in INF ϒ in TB1 and TB2 tubes.

Evidence based on clinical trials

To investigate the sensitivity of the new interferon-gamma release assay (IGRA), QuantiFERON-TB Gold Plus (QFT-Plus) (TB Sure®) was tested for active TB (used as a surrogate for latent tuberculous infection) in a Zambian TB clinic. Consecutive smear or Xpert MTB/RIF positive adult (age over 18 years) pulmonary TB patients were recruited between June 2015 and March 2016. Venous blood was tested using QFT-Plus (TB Sure®). The sensitivity was defined as the number positive divided by the total number tested. Using logistic regression, factors associated with positive QFT-Plus (TB Sure®) results were explored. Of 108 patients (median age 32 years, interquartile range 27 - 38; 73% male; 63% human immunodeficiency virus [HIV] positive), 90 were TB Sure®, 11 were negative and seven had indeterminate results; sensitivity was 83% (95%CI 75 - 90). There was no difference in sensitivity by HIV status (HIV-positive 85%, 95%CI 75 - 93; n ¼ 68 vs. HIV negative 80%, 95%CI 64 - 91; n ¼ 40; P ¼ 0.59). In models adjusted for age alone, CD4 cell count, 100 cells/ll (OR 0.15, 95%CI 0.02 - 0.96; P¼ 0.05) and body mass index ,18.5 kg/m^2 (OR 0.27, 95%CI 0.08-0.91; P ¼ 0.02) were associated with decreased odds of positive TB Sure® results [7,8].

In Vietnam, of 222 children with available QFT-Plus (TB Sure®) results, 33 were classified as confirmed TB, of whom 18 had QFT-Plus (+) and 15 had QFT-Plus (-). Multiple logistic regression modeling suggested that age, history of TB, and confirmed TB were significantly associated with having a positive TB Sure® result with an area under the ROC curve of 0.77. TB Sure® sensitivity in PTB only, EPTB, and both PTB and EPTB patients was 84.2%, 14.3% and 14.3%, respectively. The overall sensitivity of the TB Sure® (regardless PTB or EPTB) in children was 54.5% [9].

In Japan 99 patients with laboratory-confirmed active TB (patients) and 117 healthy volunteers with no risk of TB infection (controls) were studied. Blood samples were collected from both the patients and controls and tested using three types of IGRAs: the QFT-Plus (TB Sure®), the QuantiFERON-TB Gold In-Tube (QFT-GIT), and the T-SPOT.TB (T-SPOT). The sensitivity of the QFT-Plus (TB Sure®) was 98.9% (95% confidence interval [CI], 0.934 - 0.998) and similar to that of the QFT-GIT (97.9%; 95% CI, 0.929 - 0.998) and T-SPOT (96.9%; 95% CI, 0.914 - 0.994). The specificity of the QFT-Plus® was the same as that of the QFT-GIT and T-SPOT (98.1%; 95% CI, 0.934 - 0.998). One patient with uncontrolled diabetes mellitus showed negative results on all three IGRAs [10].

QFT-Plus (TB Sure®) is a new fourth generation IGRA that includes a set of peptides designed to stimulate *M. tuberculosis*-specific CD8+ T-cells. The new test shows a high specificity and retains the same sensitivity as the previous version. In addition, the increased interferon-γ release by combined stimulation of CD4+ and CD8+ T-cells observed in the newly added antigen tube (TB2) might be advantageous for improving the assay's accuracy in patients with low CD4+ T-cell counts. Increased sensitivity is a consequence of the ability of TB2 to induce a CD8 T-cell response which is mainly associated with active TB. This assay has the potential to be very useful in conditions of immune depression due to CD4 T-cell impairments.

Clinical diagnostic approach when suspecting TB in a patient

When a clinician is confronted with a patient showing some symptoms and signs of tuberculosis, from diagnostic stand point, the first question the clinician needs to address is whether it is possible to localise the site of infection and collect and submit a sample likely to contain the pathogen. If the answer is in the affirmative, then submitting the sample for Gene Xpert and MGIT culture (Gene Xpert combo) is likely to yield the highest positive diagnostic result. In 24 hours time the clinician would know whether the sample contains *M tb* DNA or not and if the sample is positive, is there Rifampicin resistance (MDR TB) or not, so that appropriate treatment can be quickly initiated. In case of pauci-bacillary infection, it is prudent to always add MGIT culture, which would take time but would make isolate available for addition drug susceptibility tests. If the sample is AFB positive, WHO recommends Line Probe Assay (LPA) one for MDR TB and LPA 2 for XDR TB. LPA would also have the ability to detect MOTT bacilli, while Gen Xpert can only detect *M tb*.

When to use TB Sure® test

If however, it is not possible to either localise the site of infection and / or collect and submit relevant clinical sample likely to contain the pathogen, submitting of 4 ml of peripheral blood in lithium heparin tube could be probed by TB Sure® test to identify if the patient is healthy with no immunological evidence of tubercular infection, or is suffering from Latent TB infection (LTBI) or has immunological evidence of recent exposure and/or active infection with *M tb* multiplying within infected macrophages leading to stimulation of TB specific CD 8+ cytotoxic T cells (as evidenced by increase in Interferon ν production in TB 2 tube.

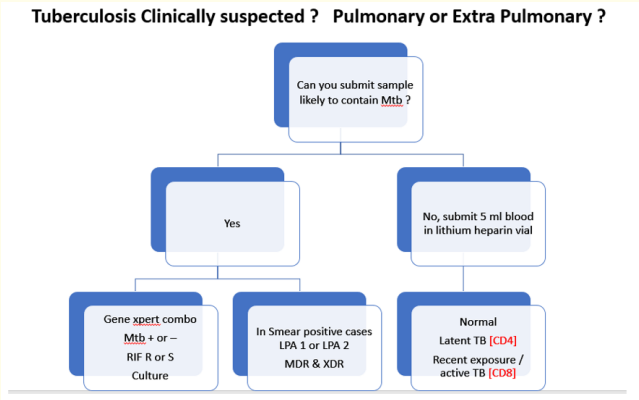
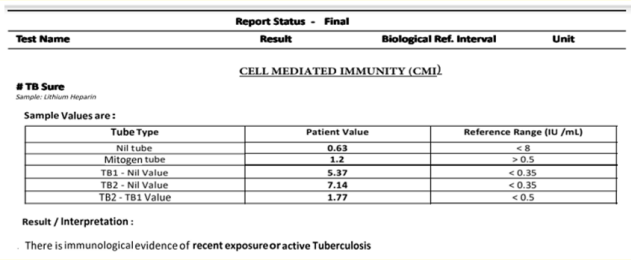


Figure 8: Suggested Algorithm when deciding what test to use in patients suspected to be suffering from Tuberculosis.

Ideally the Nil tube should show low level of pre existing Interferon ν. The mitogen tube must elicit atleast > 0.5 IU/ml INF - ν response indicating that the lymphocytes are alive and reactive. If TB 1 and/or TB 2 tube containing two specific antigens of TB show >0.35 IM/ml activity, it means that patient has been exposed to *M tb*. If TB 2 activity is more than TB 1 activity by > 0.5 IU/ml, it means that TB specific CD 8+ cytotoxic cells are active in response to multiplication of intracellular *M tb* within the macrophages.



Report Status - Final

Test Name	Result	Biological Ref. Interval	Unit

CELL MEDIATED IMMUNITY (CMI)

TB Sure
Sample: Lithium Heparin

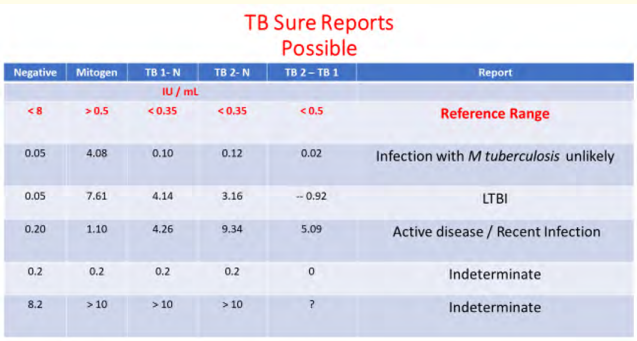
Sample Values are :

Tube Type	Patient Value	Reference Range (IU /mL)
Nil tube	0.63	< 8
Mitogen tube	1.2	> 0.5
TB1 - Nil Value	5.37	< 0.35
TB2 - Nil Value	7.14	< 0.35
TB2 - TB1 Value	1.77	< 0.5

Result / Interpretation :

There is immunological evidence of recent exposure or active Tuberculosis

Figure 9: A typical positive report.



TB Sure Reports Possible

Negative	Mitogen	TB 1- N	TB 2- N	TB 2 – TB 1	Report
		IU / mL			
< 8	> 0.5	< 0.35	< 0.35	< 0.5	Reference Range
0.05	4.08	0.10	0.12	0.02	Infection with *M tuberculosis* unlikely
0.05	7.61	4.14	3.16	-- 0.92	LTBI
0.20	1.10	4.26	9.34	5.09	Active disease / Recent Infection
0.2	0.2	0.2	0.2	0	Indeterminate
8.2	> 10	> 10	> 10	?	Indeterminate

Figure 10: Reference Ranges and four different possible results.

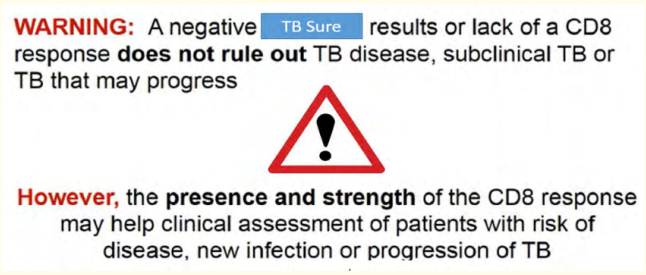
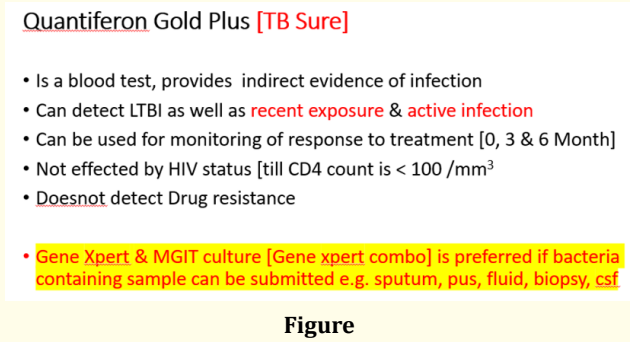


Figure 11: Caution regarding its positive predictive value.

Can TB Sure® be used for monitoring response to treatment

There is evidence that monitoring of TB specific CD 8+ cell activity can also be used for monitoring of response to treatment. It was shown in 2013 using FACS that TB specific CD 8+ cell activity decreases on a few months of successful treatment. Subsequently it has been shown that in Italy, TB therapy significantly decreased IFN-γ values and number of responders to TB1- and TB2- peptides stimulation in both LTBI and active TB patients. Stratifying LTBI subjects according to the type of preventive TB therapy used, it was found that INH treatment significantly decreased IFN-γ production. Stratifying the active TB patients according the microbiological status, it was found that TB therapy significantly decreased IFN-γ response to antigen present in QFT-Plus (TB Sure®) test in patients with clinical diagnosis compared to those with a microbiological diagnosis. In conclusions, it has been demonstrated that TB therapy decreases IFN-γ level in response to antigen present in QFT-Plus (TB Sure®) test in LTBI and active TB patients. Future studies are needed to better characterize Mtb-specifc response as a potential marker for monitoring TB therapy and preventive treatment effects [11,12].



Figure

Can TB Sure® be useful for contact tracing

Since TB Sure® has two specific antigens of *M tb* (ESAT 6 and CFP 10) and detects both LTBI by detecting TB specific CD 4+ activity and recent exposure by detecting specific CD 8+ activity, it is the best test available for contact tracing when there is history of being exposed to an open case of tuberculosis [13].

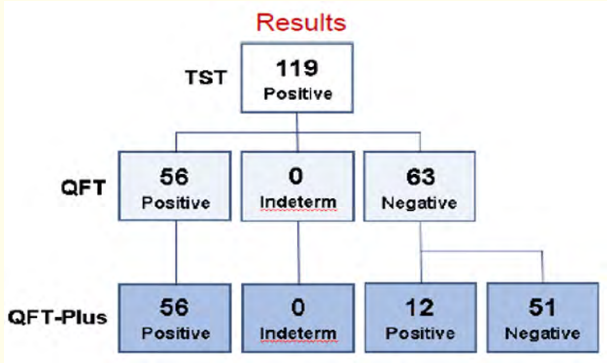


Figure 12: Results of contact tracing study [14].

Bibliography

1. World Health Organization Global Tuberculosis Report Executive Summary (2018).

2. Miguel A Arroyo-Ornelas., *et al*. Immune Diagnosis of Tuberculosis Through Novel Technologies.

3. Rozot V., *et al*. "Combined Use of Mycobacterium tuberculosis-Specific CD4 and CD8 T-Cell Responses Is a Powerful Diagnostic Tool of Active Tuberculosis". *Clinical Infectious Diseases* 60.3 (2015): 432-437.

4. Rozot V., *et al*. "Mycobacterium tuberculosis-specific CD8+ T cells are functionally and phenotypically different between latent infection and active disease". *European Journal of Immunology* 43.6 (2013): 1568-1577.

5. Petruccioli E., *et al*. "Analytical evaluation of QuantiFERON-Plus and QuantiFERON- Gold In-tube assays in subjects with or without tuberculosis". *Tuberculosis* 106 (2017) 38-43.

6. Pieterman E D., *et al*. "A multicentre verification study of the QuantiFERON®-TB Gold Plus assay". *Tuberculosis* 108 (2018) 136-142.

7. Gen Li., *et al*. "Evaluation of a New IFN-g Release Assay for Rapid Diagnosis of Active Tuberculosis in a High-Incidence Setting". *Frontiers in Cellular and Infection Microbiology* 7 (2017): 117.

8. Telisinghe L., *et al*. "The sensitivity of the QuantiFERON -TB Gold Plus assay in Zambian adults with active tuberculosis". *International Journal of Tuberculosis and Lung Disease* 21.6 (2017): 690-696.

9. Nguyen DT., *et al*. "Sensitivity and characteristics associated with positive QuantiFERON-TB Gold-Plus assay in children with confirmed tuberculosis". *PLoS ONE* 14.3 (2019): e0213304.

10. Lina Yi., *et al*. "Evaluation of QuantiFERON-TB Gold Plus for Detection of Mycobacterium tuberculosis infection in Japan". *Nature Scientific Reports* (2016): 6:30617.

11. Petruccioli E., *et al*. "Effect of therapy on Quantiferon- Plus response in patients with active and latent tuberculosis Infection". *Nature Scientific Reports* 8 (2018):15626.

12. Kamada A and Amishima M: "QuantiFERON-TB® Gold Plus as a potential tuberculosis treatment monitoring tool". *European Respiratory Journal* 49.3 (2017): 1601976.

13. Theel ES., *et al*. "Comparison of the QuantiFERON-TB Gold Plus and QuantiFERON-TB Gold In-Tube Interferon-γ Release Assays in Patients at Risk for Tuberculosis and in Healthcare Workers". *Journal of Clinical Microbiology* 56.7 (2018): JCM.00614-18.

14. Barcellini L., *et al*. "First evaluation of QuantiFERON-TB Gold Plus performance in contact screening". *European Respiratory Journal* 48.5 (2016): 1411-1419.

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