Indonesian Journal of Medicine (2021), 06(03): 298-306
Masters Program in Public Health, Universitas Sebelas Maret

Research

The Agreement of Immunoglobulin Gamma Release Assay (IGRA)/ T-SPOT Tuberculosis and Tuberculin Skin Test to Detect Latent TB Infection in Diabetes Mellitus Patients

Reviono[1)], Yusup Subagio Sutanto[1)], Harsini[1)], Leli Saptawati[2)], Marwoto[2)], Betty Suryawati[2)], Dhani Redhono[3)], Pribadi M Sebayang[1)]

[1)]Department of Pulmonology and Medical Respiratory, Faculty of Medicine, Universitas Sebelas Maret/ Dr. Moewardi Hospital, Surakarta, Indonesia
[2)]Department of Microbiology, Faculty of Medicine, Universitas Sebelas Maret / Dr. Moewardi Hospital, Surakarta, Indonesia
[3)]Department of Internal Medicine, Faculty of Medicine, Universitas Sebelas Maret / Dr. Moewardi Hospital, Surakarta, Indonesia

ABSTRACT

Background: WHO identifies diabetes mellitus (DM) as a neglected risk factor for tuberculosis (TB). Currently, there is no gold-standard test for latent TB infection (LTBI). Centers for Disease Control and Prevention (CDC) recommends using Tuberculin Skin Test (TST) and Immunoglobulin Gamma Release Assay (IGRA) to diagnose LTBI. TST is an LTBI classic diagnostic tool that has low sensitivity and specificity. But it is still preferred in diagnosing LTBI due to its lower price, and more health facilities can perform the examination compared to IGRA (T-SPOT.TB). The study aimed to measure the agreement of TST and T-SPOT.TB testing in detecting LBTI in DM and the correlation of HbA1c with TST and T-SPOT.TB.

Subjects and Method: Subjects were DM patients who underwent TST and T-SPOT.TB testing. If the results of TST and T-SPOT.TB was positive, the test would be continued with Xpert MTB/RIF microbiological testing. TST used PPD RT23 2TU. T-SPOT.TB was performed toward peripheral blood mononuclear cells. The degree of agreement between TST and T-SPOT.TB testing was calculated using the Test of Agreement (Kappa Cohen). The degree of correlation between the two variables was calculated using Pearson correlation.

Results: The selected 30 study subjects with DM undergoing antidiabetic therapy showed 6 (20%) detected LTBI and 24 (80%) without LTBI using TST and T-SPOT.TB test. There was a substantial agreement level between TST and T-SPOT.TB testing in detecting LTBI among diabetes mellitus patients undergoing antidiabetic therapy with kappa value= 0.62 ($p<0.001$). HbA1c increased T-SPOT ($r= 0.07$; $p= 0.716$) and TST ($r= 0.11$; $p= 0.956$).

Conclusion: TST testing may substitute T-SPOT.TB to detect LTBI among diabetes patients undergoing antidiabetic therapy.

Keywords: latent tuberculosis infection, diabetes mellitus, TST, Immunoglobulin Gamma Release Assay (IGRA), T-SPOT.TB

Correspondence:
Reviono. Department of Pulmonology and Medical Respiratory, Faculty of Medicine, Universitas Sebelas Maret/ Dr. Moewardi Hospital, Surakarta, Indonesia. Email: reviono@staff.uns.ac.id.

Cite this as:
Reviono, Sutanto YS, Harsini, Saptawati L Marwoto, Suryawati B, Redhono D, Sebayang PM (2021). The Agreement of Immunoglobulin Gamma Release Assay (IGRA)/ T-SPOT Tuberculosis and Tuberculin Skin Test to Detect Latent TB Infection in Diabetes Mellitus Patients. Indones J Med. 06(03): 298-306. https://doi.org/10.26911/theijmed.2021.06.03.07.

BACKGROUND

Tuberculosis (TB) is the major respiratory infection worldwide and caused 1.5 million deaths in 2014. World Health Organization (WHO) annually publishes a global TB report since 1997. The report's main purpose is to give the recent comprehensive data related to TB epidemic assessment and the improvement in its global, regional, and inter-states prevention, diagnosis, and treatment according to the recommended strategies and targets of WHO. Reducing the TB burden has been becoming a focus during the last decade and has improved in global target 2015. The result is regulated according to the targets of Millennium Development Goals (MDGs) (WHO, 2020).

Latent tuberculosis infection (LTBI) is characterized by immune response toward *Mycobacterium tuberculosis* infection without active TB clinical evidence, in which presumably one-third of the world population is infected (Getahun et al., 2015). Diabetes mellitus is a non-communicable disease that may disrupt the patients' immunity and cause susceptibility toward various infectious diseases, including TB (Martinez et al., 2014). The previous cohort study stated that DM is related to elevated TB infection risk 2-3 times. DM prevalence escalation increases the concern that the epidemic may obstruct TB infection control efforts worldwide (Pan et al., 2015; Alvarez et al., 2014)

Diabetes mellitus correlates with active TB; however, it is not definite whether DM patients have a higher risk of getting infected with LTBI. The newest WHO guidelines about LTBI management mention that systematic testing for latent TB infection among DM patients are not yet recommended (Getahun et al., 2015; Viney et al., 2015). The tuberculin skin test is a classic LBTI diagnostic tool that has low sensitivity and specificity. The low sensitivity of TST is due to the high prevalence of anergy among diabetes mellitus patients. Meanwhile, the low specificity is due to the possibility of false positives among patients who obtained *the Bacille Calmette-Guerin* (BCG) vaccine. IGRA examination has advantages compared to TST. It is more specific since it does not inflict cross-reaction toward BCG vaccine and tuberculosis mycobacterium infection. TST text becomes a preference in diagnosing LTBI because it is more economical, and more health facilities can perform the test compared to IGRA examination (Smiirnoff et al., 1998).

The value of HbA1c determined the result of controlled and uncontrolled DM, which is presumably related to LTBI immunodiagnostic examination that encourages the study (Smiirnoff et al., 1998; Hensel et al., 2016). It is discovered that the studies concerning the correlation between the value of HbA1c and the agreement of TST and T-SPOT.TB testing in detecting LTBI in DM is not yet conducted in Indonesia. The previous epidemiology studies toward DM and LTBI are still minimal and inconsistent results.

SUBJECTS AND METHOD

1. Study Design

The study used a cross-sectional design with the diagnostic test. The observational study was conducted as the subsequence of an analytical cross-sectional study to discover the correlation between the correlation HbA1c and contact history of TB patients with TST and T-SPOT.TB examination. The study was conducted in the internal medicine outpatient unit of Dr. Moewardi Hos-pital Surakarta.

2. Population and Sample

The study used the target population of DM patients who underwent antidiabetic therapy. The subjects who met inclusion criteria were subsequently given education

and data recording, including identity, anamnesis, physical examination, laboratory investigation, namely investigation, the value of HbA1c, TST, and T-SPOT.TB examination. Sub-jects were required to come again within 48-72 hours for the TST reading.

The study subjects with positive TST and T-SPOT.TB subsequently underwent Xpert MTB/RIF microbiological examination detected *Mycobacterium tuberculosis* can be excluded from the study since it was TB active. Tuberculin skin test used PPD RT23 2TU, which was produced by BIO FARMA Bandung-Indonesia. PPD RT23 2TU has intradermally injected as much as 0.1 ml into the forearm volar by using a 1 cc syringe. Results interpretation in induration was read after 72 hours, and a positive result was obtained if the induration was ≥10 mm. A total of 30 subjects were selected using purposive sampling technique based on inclusion and exclusion criteria.

3. Study Variables

The dependent variable was latent tuberculosis infection (LTBI). The independent variables were the Tuberculin skin test (TST), T-SPOT.TB, and HbA1C.

4. Operational Definition of Variables

Latent TB infection (LTBI) is when a person is infected with *Mycobacterium tuberculosis* however does not inflict any TB clinical signs and symptoms (cough >2 weeks, coughing up blood, chest pain, shortness of breath, weight loss, loss of appetite, feeling weak and malaise, and night sweat), and normal thorax x-ray image. But, the results of immunological tests such as TST and IGRA are positive without symptoms and clinical signs of active both pulmonary and extrapulmonary TB (lymphadenitis TB, pleuritis TB, and renal TB).

Tuberculin Skin Test is an examination that measures the cellular immune response of delayed-type hypersensitivity (DTH) toward purified protein derivative in patients who were previously infected with *Mycobacterium tuberculosis*. It is injected intracutaneously into the forearm volar. Induration is measured 48-72 hours after the injection by palpating the induration. The cut-off value used is the induration diameter of ≥5 mm.

T-SPOT.TB measures the amount of IFN-γ produced by T-cell in responding to ESAT-6 and CFP-10 antigen and is based on enzyme-linked immunosorbent spot (ELISPOT) assay. The measuring method enumerates the spots inside panel A (ESAT-6) and panel B (CFP-10) wells. The result is positive if it obtained spot forming units (SFUs) on antigen M. tuberculosis panel A (ESAT-6) and/or panel B (CFP-10) ≥8.

Haemoglobin A1c (HbA1c) is the minor Component of Hb that binds with glucose for DM diagnosing and long-term control. Measuring is conducted using reverse-phase cation exchange chromatography based on High-Performance Liquid Chromatography (HPLC) and Capillary electrophoresis from the Serbia system. It is stated as DM when the result is ≥6.5%

5. Study Instruments

T-SPOT.TB examination was conducted toward peripheral blood mononuclear cells separated through a process by using centrifuge out of peripheral vein blood as much as 8 ml. The test consisted of two antigen wells containing *Mycobacterium tuberculosis* ESAT-6 and CFP10 specific antigen, *Mycobacterium tuberculosis*. The positive control contained *phytohaemagglutinin*, and the control negative did not contain mitogen nor antigen. The disc was incubated all night at 37°C and 5% CO_2 atmosphere. Spot-forming units (SFUs) were enumerated using an ELISPOT reader.

Xpert MTB/RIF examination was conducted by collecting sputum in sample tubes. Subsequently, it was incubated for 15 minutes at room temperature. The sample was diluted inside a cartridge by using a pipette. The cartridge was then put inside the GeneXpert machine, and the test was ready to start. The result interpretation was measured based on fluorescence signs, and the algorithm appeared on the screen.

6. Data Analysis

The agreement degree between TST and T-SPOT.TB examinations were enumerated by using Test of Agreement (Kappa Cohen) with the value of kappa: ≥0.8 (excellent agreement), kappa: 0.6–0.8 (fair agreement), and kappa: <0.6 (poor agreement). The correlation degree of the variables was tested by using the contingency coefficient.

7. Research ethics

Ethical clearance was obtained from the ethics committee of the educational hospital Dr. Moewardi Hospital Surakarta no: 681/IX/ HREC/2018

RESULTS

Characteristics of the study subjects were based on gender, age, contact history, BCG scar, dan HbA1c value is presented in Table 1. Based on the characteristics of the study subjects, the number of female subjects was 60% higher than the number of male subjects. There were more subjects from under 60 years age group (56.7%). In the study subject, only one person had the TB contact history in the last month. BCG scars were found on the majority of the subjects, which means they were once vaccinated with the TB vaccine. HbA1c examination indicated that the majority of the subjects were above 65%, which is most of the study subjects were controlled DM (70%).

Table 1. Characteristics of the Study Subjects (Categorical Data)

Variables	Total N (%)	TST (+) N (%)	T-SPOT (+) N (%)
Total	30 (100%)	3 (10.0%)	6 (20.0%)
Gender			
Male	12 (40.0%)	3 (10.0%)	4 (13.3%)
Female	18 (60.0%)	0 (0.0%)	2 (6.7%)
Age			
<60 years	17 (56.7%)	1 (3.3%)	2 (6.7%)
≥60 years	13 (43.3%)	2 (6.7%)	4 (13.3%)
Contact History			
Yes	1 (3.3%)	0 (0.0%)	1 (3.3%)
No	29 (96.7%)	3 (10.0%)	5 (16.7%)
BCG Scar			
Yes	26 (86.7%)	3 (10.0%)	5 (16.7%)
No	4 (13.3%)	0 (0.0%)	1 (3.3%)
HbA1c			
≥6.5	21 (70.0%)	3 (10.0%)	5 (16.7%)
<6.5	9 (30.0%)	0 (0.0%)	1 (3.3%)

There were three respondents (10%) who were positive for both TST and T-SPOT.TB. No respondent (0.00%) was found positive for the TST examination and negative for T-SPOT.TB. Three respondents (10%) were found negative for the TST examination

and positive for T-SPOT.TB. There were 24 respondents (80%) who were found negative for both examinations. The results are presented in Table 2.

Table 2. The Correlation Degree of TST and T-SPOT.TB Examination in Detecting LTBI among Diabetes Mellitus Patients Who Underwent Antidiabetic Therapy

T-SPOT	TST		Total	K	p
	Positive	Negative			
Positive	3	0	3	0.62	<0.001
Negative	3	24	27		
Total	6	24	30		

From the enumerating results of agreement degree, it was discovered the Kappa value was 61.5%. The above data indicated that the agreement degree K value =0.62 was substantial or the strength was sufficient (0.60<K<0.80). It means that TST examination can be used to diagnose LTBI among diabetes mellitus patients equal to T-SPOT.TB.

Correlation between HbA1c value and TST examination was tested using bivariate analysis over the overall study subjects (30 respondents). The data used in the study were on a nominal scale; therefore, the correlation test used was the nonparametrically test, namely the Pearson correlation test. The study result discovered three positives (14.28%) for the HbA1c value ≥6.5 and 18 negatives (60.0%) for diabetes mellitus patients with HbA1c ≥6.5. HbA1c increased the TST examination (r= 0.11; p= 0.956).

Table 3. Correlation between HbA1c Value and TST in LTBI Detection (N=30)

Independent Variable	r	p
HbA1c	0.11	0.956

Correlation between HbA1c value and T-SPOT examination was tested using bivariate analysis over the overall study subjects (30 respondents). The result discovered 5 (16.7%) positive T-SPOT results with HbA1c value ≥6.5 and 16 (53.3%) negative results for diabetes mellitus patients with HbA1c value >6.5. HbA1c increased T-SPOT examination (r= 0.07; p= 0.716) as it is presented in Table 4.

Table 4. Correlation between HbA1c and T-SPOT.TB in LTBI Detection

Independent variable	r	p
HbA1c	0.07	0.716

DISCUSSION

The study was a diagnostic study to discover the degree of agreement between TST and T-SPOT.TB diagnostic tools in detecting LTBI among diabetes mellitus patients who underwent antidiabetic therapy. The study discovered LTBI among diabetes mellitus patients who underwent antidiabetic therapy with a total of 20% respondents. A similar result is also reported by Meng RL et al. in Taiwan of 2017, which reported latent TB infection among diabetes patients who underwent antidiabetic therapy as much as 22.8%. In this study, T-SPOT.TB examination result indicated higher positive latent TB infection compared to TST with a comparison of 20% and 10%. Several previous studies also

showed results that are more likely similar to the result of this study in which T-SPOT.TB indicated a higher positive result than TST in detecting LTBI among diabetes mellitus patients (Meng et al., 2017). T-SPOT.TB examination has better sensitivity and specificity than TST in diagnosing LTBI among DM patients (Zhu et al., 2014).

The result of the study discovered three respondents were positive for TST and positive for T-SPOT.TB, three respondents, were negative for TST and positive for T-SPOT.TB, and 24 respondents were negative for TST and negative for T-SPOT.TB. Examination result that was positive for TST and negative for T-SPOT.TB can be generated by false-positive of TST examination. It is possibly the reactivation of previous BCG vaccination or infection of Mycobacterium Other Than Tuberculosis (MOTT). However, the study also discovered three respondents without BCG scars, therefore the positive result for TST and negative for T-SPOT.TB was not the reactivation of BCG vaccination. MOTT infection may generate false-positive results for TST examination in regions with low TB prevalence and high MOTT prevalence. Indonesia has a high TB prevalence; therefore, the negative result for TST and positive for T-SPOT.TB in the study was not false positive due to the BCG vaccine and MOTT infection; instead, it was positive with LTBI (Ayub et al., 2004). Three respondents with a negative result for TST and positive for T-SPOT.TB was presumeably generated by false negative for TST examination. However, the study obtained negative results for TST and positive for T-SPOT.TB among respondents with HbA1c value ≥6.5% therefore there was no false-negative and anergy among respondents of the study.

There were three respondents with no agreement between the result of TST dan T-SPOT.TB examination in which the results were negative for TST and positive for T-SPOT.TB. Among the three respondents with no agreement, there was one respondent aged <60 years old and two respondents aged ≥60 years old. The degree of agreement between TST and T-SPOT.TB examination as LTBI diagnosis tools was measured by using Cohen's Kappa degree of agreement. Degree of agreement between TST and T-SPOT.TB in the study was K= 0.62 with the value of p<0.001, which means it was a substantial degree of agreement (0.60<K≤0.80) and statistically significant (Reviono et al., 2019).

TST examination has several weaknesses compared to T-SPOT.TB, such as the occurrence of false-positive and false negative. However, the result of the study discovered a substantial degree of agreement between TST and T-SPOT.TB examination. It means that TST examination can be used as LTBI diagnostic tool, which is equal to T-SPOT.TB among diabetes mellitus patients, therefore TST examination is still recommended to detect LTBI among diabetes mellitus patients who undergo antidiabetic therapy since it is more practical, economical, and available in healthcare facilities.

The absence of a gold standard in LTBI diagnosis generates difficulty in determining the sensitivity and specificity of TST and T-SPOT.TB examination in detecting LTBI among diabetes mellitus patients. In this study, the researchers did not assess sensitivity and specificity values for TST diagnostic tools since we consider the proper gold standard for LTBI test is not yet available, including T-SPOT.TB, even if other researchers use T-SPOT.TB as the gold standard for LTBI diagnosis (Diel R et al., 2010).

To the researcher's knowledge a study of the correlation between HbA1c value and TST and T-SPOT.TB examination among

diabetes mellitus patients has never been conducted up to the present. We expect HbA1c examination can predict the potential LTBI. A study by Baghaei et al. (2013) mentioned a correlation between diabetes and tuberculosis as the future challenge. It requires TB screening among DM patients. WHO recommends HbA1c examination as a diagnostic test for DM (Baghaei et al., 2013).

The researchers compared it to the previous study toward DM patients that is a study by Lee et al. (2006), which stated the occurrence of correlation between uncontrolled DM and the development of TB infection into active. That study obtained the value of r= 0.23; p= 0.232, which means there is no significant correlation between HbA1c and the size of TST examination induration and weak correlation value and positive correlation direction. The result of the study is different from a study by Lee PH et al., which stated there is a correlation between uncontrolled DM and TB infection. It discovered a significant correlation between HbA1c and TB risk factors with the value (r= 0.28; p<0.001). That study has the same correlation direction that is positive correlation direction which direction, the lower the HbA1c value, the smaller the risk to get of getting TB, and vice versa. The value of HbA1c indicates one of diabetes mellitus statuses, controlled or uncontrolled. HbA1c value of less than 6.5% indicates controlled diabetes mellitus status and responds to PPD 23 RT injection by forming larger induration in TST examination (Lee et al., 2016).

The correlation test result of HbA1c value and T-SPOT.TB examination did not obtain a significant correlation with a very weak correlation value. The study result indicates a positive T-SPOT. Tuberculosis results are found more in panel B (M. tuberculosis CFP-10 antigen) than panel A (*M. tuberculosis* ESAT-6 antigen). It is possibly due to the antigenic nature of *M. tuberculosis* CFP-10, which is stronger than the *M. tuberculosis* ESAT-6 antigen. The germs strain of *M. tuberculosis* inside the respondents gives immunity and responds more toward *M. tuberculosis* CFP-10 antigen. The difference of T-SPOT.TB positivity between ESAT-6 SFUs and CFP-10 SFUs generates a different correlation direction between HbA1c value and ESAT-6 SFUs and CFP-10 SFUs. The limitation of the study is the study does not assess the antigenic nature of MTB ESAT-6 and CFP-10.

The substantial agreement degree between TST and T-SPOT.TB detecting LTBI among patients with diabetes mellitus who undergo antidiabetic therapy with kappa value was 0.62 (0.60<K≤0.80), p<0.001, TST can substitute T-SPOT.TB to detect LTBI in DM case. It discovers the correlation between TST and T-SPOT.TB toward HbA1c is an insignificant correlation. Therefore, the value of HbA1c does not correlate to the potential LTBI occurrence.

AUTHOR CONTRIBUTION

Reviono did proposal formulation. Pribadi Sebayang did clearance handling. Yusup Subagio did consumables supply. Leli Saptowati and Betty Suryawati did laboratory preparation. Harsini did the data collection. Reviono and Marwoto did the data analysis. Dhani Redono did study report formulation.

FUNDING AND SPONSORSHIP

The study was funded by the Research Institute and Community Service (LPPM) of UNS through a research group grant.

CONFLICT OF INTEREST

There is no conflict of interest since the funding donor does not involve in the research implementation.

ACKNOWLEDGEMENT

We deliver our gratitude to LPPM for the opportunity given and the Director of Moewardi General Hospital, who allowed us to use the hospital's facilities for the study.

REFERENCE

Alvarez GG, VanDyk DD, Aaron SD (2014). TAIMA (stop) TB: the impact of a multifaceted TB awareness and door-to-door campaign in residential areas of high risk for TB in Iqaluit, Nunavut. PLoS One. 9: e100975. https://doi.-org/10.1371/journal.pone.0100975.

Ayub A, Steven H, Reed KD, Nasser RM, Gilbert SR (2004). Testing for latent tuberculosis. Clin Med Res. 2(3): 191–194. https://dx.doi.org/10.3121%2Fcmr.2.3.191.

Baghaei P, Marjani M, Javanmard P, Tabarsi P, Masjedi MR (2013). Diabetes mellitus and tuberculosis facts and controversies. J Diabetes Metab Disord. 12(1): 58. https://doi.org/-10.1186/2251-6581-12-58.

Diel R, Loddenkemper R, Nienhaus A (2010). Evidence-based comparison of commercial Interferon - γ Release Assays for detecting active TB. Chest. 137(4): 952-68. https://doi.org/10.13-78/chest.09-2350.

Getahun H, Matteelli A, Abubakar I, Aziz MA, Baddeley A, Barreira D, Boon SD, et al. (2015). Management of latent *Mycobacterium tuberculosis* infection: WHO guidelines for low tuberculosis burden countries. Eur Respir J. 46(6): 1563–76. https://doi.org-/10.1183/13993003.01245-2015.

Hensel RL, Kempker RR, Tapia J, Oladele A, Blumberg HM, Magee MJ (2016). Increased risk of latent tuberculous infection among persons with pre-DM and DM mellitus. Int J Tuberc Lung Dis. 20(1): 71–8. https://dx.doi.org/-10.5588%2Fijtld.15.0457.

Lee PH, Fu H, Lai TC, Chiang CY, Chan CC, Lin HH (2016). Glycemic control and the risk of tuberculosis: A Cohort study. Plos medicine. 13(8): e100-2072. https://doi.org/10.1371-/journal.pmed.1002072.

Martinez N, Kornfeld H (2014). DM and immunity to tuberculosis. Eur J Immunol. 44(3): 617–26. https://-doi.org/10.1002/-eji.201344301.

Meng RL, Ya PH, Yu TK, Chen HL, Yun JS, Chin CS, Jun YW, et al. (2017). Diabetes Mellitus and Latent Tuberculosis Infection: A systemic review and meta analysis. Clin Infect Dis. 64(6):719-27. https://dx.doi.org/10.-1093%2Fcid%2Fciw836.

Pan SC, Ku CC, Kao D, Ezzati M, Fang CT, Lin HH (2015). Effect of DM on tuberculosis control in 13 countries with high tuberculosis: a modelling study. Lancet DM Endocrinol. 3(5): 323–30. https://doi.org/-10.1016/-s2213-8587(15)00042-x.

Reviono, Saptawati L, Redhono D, Suryawati B (2019). Good agreement between an interferon gamma release assay and tuberculin skin tests in testing for latent tuberculosis infection among HIV-infected patients in Indonesia. Korean Med Sci. 34(40):-e259 https://doi.org/10.3346-/jkms.-2019.34.e259.

Smirnoff M, Patt C, Seckler B, Adler JJ (1998). Tuberculin and anergy skin testing of patients receiving long-term hemodialysis. Chest. 113(1): 25-7.

https://doi.org/10.1378/chest.113.1.25.

Viney K, Cavanaugh J, Kienene T (2015). Tuberculosis and DM mellitus in the Republic of Kiribati: a case-control study. Trop Med Int Health. 20(5): 650–7. https://doi.org/10.1111/tmi.12462.

WHO (World Health Organization) (2020). Global Tuberculosis Report 2019. 20th Ed. [cited 2021 june 19th]. Available: http://www.who.int/iris/handle/10665/191102.

Zhu C, Liu Z, Li Z, Mei S, Hu Z (2014). The performance and limitation on T-SPOT.TB for the diagnosis of TB in a high prevalence setting. J Thorac Dis. 6(6): 713-719. https://dx.doi.org/10.3978%2Fj.issn.2072-1439.2014.04.38