TÜBİTAK

Original Article

Turk J Med Sci
2010; 40 (4): 585-591
© TÜBİTAK
E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-0907-85

The comprehensive evaluation of latent tuberculosis infection in health care workers and of patients with active tuberculosis using TST, ELISA, and ELISPOT methods

Hülya ŞİMŞEK[1], Sibel Meryem ALPAR[2], Kurtuluş AKSU[2], Funda AKSU[2], İsmail CEYHAN[1], Salih CESUR[3], Ayşegül GÖZALAN[1], Mustafa ERTEK[1]

Aim: Until recently, the tuberculin skin test (TST) has been the only assay used for detecting latent tuberculosis infection (LTBI), but two ex-vivo tests, used as alternative methods to TST, based on enumerating the M. tuberculosis-specific interferon (IFN)-γ response are now commercially licensed. The aim of this study was to compare the sensitivity and specificity of TST, QuantiFERON Gold (QFT-G), and T-SPOT.TB in diagnosing LTBI and active tuberculosis (TB).

Materials and methods: This study was carried out with 95 participants including 3 groups (negative control, close contact, and patient groups) during a 10-month period from March 2007 to January 2008.

Results: When the cut-off value of the TST was regarded as ≥15 mm, 46.4% of the patients and 14.3% of the control group were found to have positive values. The sensitivity (51.4%) and the negative predictive value (NPV) (52.6%) of TST were lower than the specificity (83.3%) and the positive predictive values (PPV) (82.6%). The sensitivity and the PPV of the QFT-G test (78.4% and 76.3%, respectively) were higher than the specificity (62.5%) and NPV (65.2%). The PPV (81.8%) of the T- SPOT.TB test was higher than sensitivity (73.0%), specificity (75.0%), and NPV (64.3%).

Conclusion: IFN-γ tests could be useful in diagnosing LTBI and chemo-prophylaxis, as the false negativity of the TST was higher compared to both QFT-G and T-SPOT.TB. However, additional studies are needed to assess better the utility of these tests with large populations.

Key words: IFN-γ tests, QFT-G, T-SPOT.TB test, tuberculin skin test (TST), latent TB

Hastane çalışanlarında latent tüberküloz enfeksiyonunun ve aktif tüberkülozlu hastaların TDT, ELISA ve ELISPOT yöntemleriyle kapsamlı değerlendirilmesi

Amaç: Son zamanlara kadar, Tüberkülin Deri Testi (TDT) Latent tüberküloz enfeksiyonunu (LTBI) saptamada kullanılan yegane testti, fakat şimdi TDT'ye alternatif metot olarak M. tuberculosis'e özgü interferon (IFN) - γ cevabını ölçmeye dayalı 2 ex-vivo test ticari olarak ruhsatlandırılmıştır. Bu çalışmada, aktif tüberkülozun ve LTBI'nin tanısı için TDT, QuantiFERON Gold (QFT-G) ve T-SPOT.TB testlerinin özgüllük ve duyarlılıklarının karşılaştırılması amaçlanmıştır.

Yöntem ve gereç: Bu çalışma Mart 2007 ile Ocak 2008 tarihleri arasında 10 aylık bir dönemde 3 gruptan (negatif kontrol, yakın temaslı ve hasta grubu) oluşan 95 katılımcıyla gerçekleştirilmiştir.

Bulgular: TDT'nin eşik değeri ≥15 mm sayıldığında, hastaların % 46,4'ü ve kontrol grubunun % 14,3'ünde pozitif değerler bulunmuştur. TDT'nin duyarlılığı (% 51,4) ve Negatif Prediktif Değeri (NPV) (% 52,6), seçiciliği (% 83,3) ve Pozitif Prediktif Değerinden (PPV) (% 82,6) daha düşüktü. QFT-G testinin duyarlılığı ve PPV'si (sırasıyla % 78,4 ve % 76,3), seçicilik (% 62,5) ve NPV'sinden (% 65,2) daha yüksekti. T-SPOT.TB testinin PPV'si (% 81,8) duyarlılık (% 73,0), seçicilik (% 75,0) ve NPV'sinden (% 64,3) daha yüksekti.

Received: 09.07.2009 – Accepted: 10.02.2010

[1] Refik Saydam National Public Health Agency, Ankara - TURKEY
[2] Atatürk Chest Disease and Thoracic Surgery Center, Ankara - TURKEY
[3] Ankara Research and Training Hospital, Ministry of Health, Ankara - TURKEY
Correspondence: *Hülya ŞİMŞEK, Refik Saydam National Public Health Agency, National Tuberculosis Reference and Research Laboratory, Ankara - TURKEY*
E-mail: hsimsek_tr@yahoo.com

Sonuç: TDT'nin yanlış negatifliği QFT-G ve T-SPOT.TB testlerinden daha yüksek olduğu için IFN-γ testlerinin LTBI tanısında ve hastalığın önlenmesinde faydalı olabileceği sonucuna varıldı. Yine de geniş popülasyonda bu tür testlerin kullanırlılığını daha iyi değerlendirmek için ilave çalışmalara ihtiyaç vardır.

Anahtar sözcükler: IFN-γ testleri, QFT-G, T-SPOT.TB testi, tüberkülin deri testi (TDT), latent TB enfeksiyonu

Introduction

Tuberculosis (TB) is an important health problem due to the difficulties in disease control and its increasing frequency worldwide, especially in Turkey. The World Health Organization (WHO), in an attempt to raise public and political awareness in 1993, declared that TB was a global emergency. It is estimated that, from 2000 to 2020, nearly 1 billion people will be newly infected, 200 million will get sick, and 35 million will die from TB if control measures are not significantly improved (1). In 2007, the Department of TB Control reported that TB incidence was 26 per 100,000 in Turkey (2).

Despite the increasing global burden, there is still no reliable test to confirm active *M. tuberculosis* infection in culture-negative cases, or to detect latent infection in asymptomatic individuals, because TST is of limited sensitivity and specificity (3,4).

In the TB elimination strategies, chemotherapy is important to prevent LTBI before it progresses. Those who carry LTBI are at greater risk of reactivation of TB for many years. Until recently, TST was used in diagnosing LTBI (5-7). It is known that TST lacks sensitivity; particularly in HIV-infected individuals, it has poor specificity because of antigenic cross-reactivity with Bacillus Calmette-Guérin (BCG) vaccination and also in the population in which nontuberculous mycobacteria (NTM) incidence is high. ESAT-6 and CFP-10 are antigens expressed in *M. tuberculosis*, but not in *M. bovis* BCG and most NTM (8,9).

There are 2 commercially available antigen-specific IFN-γ assays for the management of TB (10). A whole-blood enzyme-linked-immunosorbent assay (ELISA) that measures IFN-γ concentrations in supernatants (QFT-G -TB GOLD; Cellestis Ltd., Australia) has been evaluated in a routine hospital setting recently. Notably, over 20% of the results were indeterminate as a result of the failure of the mitogen-driven positive control, and this was significantly associated with iatrogenic immunosuppression 11). An enzyme-linked immunospot assay (ELISPOT) that detects individual IFN-γ-producing T cells (T-SPOT.TB; Oxford Immunotec, Oxford, UK) had low technical failure rates and high diagnostic sensitivity in HIV-infected adults and children with active TB (12-14).

In this study, it was aimed to determine the validity of TST and 2 commercially available tests by enumerating the *M. tuberculosis*-specific IFN-γ response to display the LTBI presence in individuals with a positive history of close contact with active TB patients and health workers as well as individuals with active TB due to the high incidence of BCG vaccination and LTBI in Turkey.

Materials and methods

Study population: A total of 95 individuals participated in this study during a 10-month period from March 2007 to January 2008. Participants were grouped according to their infection-risk: group 1, patients with active pulmonary TB diagnosed through clinical examination, chest X-ray, Acid-Fast Staining (AFS), and culture at Atatürk Chest Diseases & Thoracic Surgery Center (n = 37); group 2, health care workers at TB clinics and some TB patients' relatives accompanying them (n = 34); group 3, no identifiable risk of *M. tuberculosis* infection (n = 24). All participants had negative HIV results. The study was approved by the local ethics committee. Both written and verbal informed consents were obtained from the subjects and they were questioned regarding the BCG vaccination and history of close contact with a TB patient.

Tuberculin Skin Test (TST): For the TST, 0.1 mL from 5 TU of PPD-S (BD-NCIPD Ltd. Sofia) was injected intradermally into the volar side of the forearm (2/3 upper side) by the Mantoux method.

Transverse induration diameter was measured by the ballpoint method 72 h later, and TST results were interpreted according to the induration diameters (15,16).

T-SPOT.TB Test: Between 0700 and 0800, 8 mL venous blood samples were drawn from the participants and the samples were studied the same day within the following 2 h. The T-SPOT.TB test study was performed according to the manufacturer's instructions. For this test, 250,000 peripheral blood mononuclear cells (for per well)/100 µL AIM-V medium pipetted into 4 wells were used for every person from a 96-well microtiter plate precoated with monoclonal antibodies directed against IFN- . After incubation for 16-20 h at 37 °C in a CO_2 incubator, the plate was washed with phosphate-buffered saline solution (PBS) and incubated with conjugate. The reaction was stopped following the substrate. After the test wells dried, they were scored as either positive or negative (17).

QuantiFERON-TB Gold test: QFT-G in-tube assay was performed according to the instructions included in the QFT-G (CSL Ltd) assay kit. Venous blood from every subject was collected and put into 3 different 1 mL-heparin containing tubes (Nil, Antigen, and Mitogen). Within 6 to 8 h after the blood was drawn, the tubes were incubated for 16-24 h at 37 °C. Following centrifugation, blood samples were kept under cold storage conditions until they were tested. IFN- amounts secreted from plasma were measured with the ELISA method. The test results were determined as either negative or positive, using the QFT-G analysis software of the manufacturer (18).

Statistical analysis: Statistical analyses were carried out using SPSS version 15.0. To compare the groups, Student's t test for independent samples was applied for the continuous variables, and the chi-square test for the categorical variables.

Results

Of the total 95 recruited subjects in the study group, 37 (38.9%) were patients with active pulmonary TB, 24 (25.3%) were healthy controls, and 34 (35.8%) were close contacts who were healthcare workers at TB clinics and some TB patients' relatives accompanying them. The median age of the study population was 42.20 ± 14.42 (min. = 12, max. = 79), and 56 (58.9%) were men, while 39 (41.1%) were women. TST induration diameters of the 78 subjects (81.1%) were ≥10 mm, and those of 33 subjects (34.7%) were ≥15 mm. The values of positive QFT-G test, T-SPOT.TB test, and TST in all groups were 61.1%, 55.8%, and 38.9%, respectively.

Table 1 shows the distribution of the subjects according to the test results of the 3 groups in the study. Out of 34 participants in the close contact group, 32 were BCG-vaccinated, whereas only 2 were

Table 1. Comparison of the results of 3 tests used in the study according to BCG status and the subjects.

		TST* Positive	QFT-G Positive	T-SPOT.TB Positive
Patient	BCG (+)	13/ 28 (46.4%)	23/28 (82.1%)	20/28 (71.4%)
	BCG (-)	6/ 9 (66.7%)	6/9 (66.7%)	7/9 (77.8%)
	Total	19/37 (51.5%)	29/37 (78.4%)	27/37 (73.0%)
Control	BCG (+)	3/ 21 (14.3%)	8/21 (38.1%)	6/21 (28.6%)
	BCG (-)	1/ 3 (33.3%)	1/3 (33.3 %)	-
	Total	4/24 (16.7%)	9/24 (37.5%)	6/24 (25%)
Contact	BCG (+)	13/ 32 (40.6%)	19/32 (59.4%)	19/32 (59.4%)
	BCG (-)	1/ 2 (50.0%)	1/2 (50.0%)	1/2 (50.0%)
	Total	14/34 (41.2%)	20/34 (58.8%)	20/34 (58.8%)
	General total	37/95 (38.9%)	58/95 (61.1%)	53/95 (55.8%)

Cut-off value of TST positivity is ≥15 mm with BCG history, and ≥10 mm without BCG history

non-vaccinated. Thirteen of 32 vaccinated-contacts had 2 or 3 BCG scars. According to the vaccination status, the results of QFT-G and T-SPOT.TB tests were similar; the number of BCG scars did not affect the results of QFT-G and T-SPOT.TB. The number of close contact participants with both QFT-G and T-SPOT.TB positivity was equal (58.8% for each), while TST positivity was lower than that of the 2 tests (41.2%). In 2 contacts with QFT-G negative and T-SPOT.TB positive, the result of either ESAT-6 or CFP10 antigen for T-SPOT.TB test was positive; in other words one of the antigens was negative.

Out of the 24 healthy controls, 21 were BCG-vaccinated and 3 were non-vaccinated. When the results of 3 tests for the control group were evaluated, it was found that the values of the positivity of QFT-G, T-SPOT.TB, and TST were 37.5%, 25.0%, and 16.7%, respectively. This was considered as the false positiveness of the 3 tests for the control group.

In terms of QFT-G, T-SPOT.TB, and TST data, the positivity of the tests was found to be 6.04 (95% CI: 1.94-18.86), 8.10 (95% CI: 2.50-26.22), and 5.33 (95% CI: 1.35-21.06) times higher in the patient group compared to the control group, respectively.

Table 2 shows that there is no statistically significant difference between groups in terms of gender, age, and BCG status. There is a statistically significant difference between control and the patient group for QFT-G and T-SPOT.TB (P = 0.001 and P = 0.001, respectively).

The validity was calculated using culture results as the reference method for QFT-G, T-SPOT.TB, and TST, but the contact group was excluded from validity tests. When, all in all, the culture positive-patient group and the culture negative-control group were considered as reference, the specificities of QFT-G (62.5%) and PPV (76.3%) were found lower than those of T-SPOT.TB and TST.

The sensitivities of T-SPOT.TB test (73.0%) and NPV (64.3%) were lower than that of QFT-G.

Sensitivities of TST (51.4%) and NPV (52.6%) were the lowest 2 values. TST also yielded false negative results. Specificities of TST (83.3%) and PPV (82.6%) were high (Table 3).

Table 2. Distributions of the groups according to gender, age, TB history, and BCG status of QFT-G and T-SPOT.TB test results.

	QFT-G			T-SPOT.TB		
	Positive (n = 58)	**Negative (n = 37)**	**P**	**Positive (n = 53)**	**Negative (n = 42)**	**P**
Gender						
Male (n, %)	38 (67.9)	18 (32.1)	0.103	32 (57.1)	24 (42.9)	0.750
Female (n, %)	20 (51.3)	19 (48.7)		21 (53.8)	18 (46.2)	
Age (years)	43.4 ± 13.7	40.4 ± 15.6	0.328	43.7 ± 15.1	40.4 ± 13.4	0.270
TB history						
Control (n, %)	Ref.*			Ref.*		
Patient (n, %)	29 (78.4)	8 (21.6)	0.001	27 (73.0)	10 (27.0)	0.001
Contact (n, %)	20 (58.8)	14 (41.2)	0.110	20 (58.8)	14 (41.2)	0.110
BCG status						
Negative (n, %)	8 (57.1)	6 (42.9)	0.745	8 (57.1)	6 (42.9)	0.912
Positive (n, %)	50 (61.7)	31 (38.3)		45 (55.6)	36 (44.4)	

Data are presented as mean ± SD, unless otherwise stated.
*Ref. Reference group

Table 3. The validity test results of TST, QFT-G, and T-SPOT.TB.

	Reference Test-Sputum culture		
	QFT-G (%)	**T-SPOT.TB (%)**	**TST* (%)**
Sensitivity (%)	78.4	73.0	51.4
Specificity (%)	62.5	75.0	83.3
PPV (%)	76.3	81.8	82.6
NPV (%)	65.2	64.3	52.6

* Cut-off value of TST positivity is ≥15 mm with BCG history and ≥10 mm without BCG history (PPV: Positive Predictive Value; NPV: Negative Predictive Value).

Discussion

Positive TST may either depend on *M. tuberculosis* infection or show the positivity related to BCG vaccination or NTM. It is very hard to discriminate these results from the responses for *M. tuberculosis* and it is a risk to choose the positive value in subgroups. Additionally their sensitivity may decrease in immunocompromised people depending on the anergy, and it may cause many problems, such as false readings, differences between readers, need for educated personnel, longer evaluation time, and the need to refer to different cut-off values for different populations.

The determination of ESAT 6 and CFP 10 antigens is a great improvement in the diagnosis of active TB and LTBI, as well as in the discrimination between *M. tuberculosis* infection and former BCG vaccination (18).

In the present study, the validities of QFT-G and T-SPOT.TB tests were determined by enumerating IFN-γ and TST by accepting positive-mycobacterium culture as reference. Since it is well known that responses to IFN-γ tests may change in TB patients after they are put on antituberculous chemotherapy, only newly diagnosed TB patients who had no prior therapy were enrolled in the study (19,20). In the whole study population, no significant relationship of the positive results with gender or age was observed. When the TST cut-off value was accepted as induration ≥15 mm in vaccinated subjects and 10 mm in non-vaccinated subjects based on the literature, it was shown that TST was able to correctly diagnose 51.4% of the patients. Because of the high level of false-negative TST, its sensitivity was low. However, true-negativity in the control group was 83.3%, and this percentage was higher than those of QFT-G (62.5%) and T-SPOT.TB (75.0%) tests.

QFT-G test detected more patients with active TB than TST and T-SPOT.TB test. The sensitivity of QFT-G test was 78.4% and its PPV was 76.3%. In conclusion, this test was more successful in differentiating patients than other tests. However, QFT-G test might have false positive results.

Similarly, Miyashita et al. showed that QFT-G test was more successful than TST in diagnosing patients. It was inferred that the QFT-G test could accurately detect TB infections and was useful in the contact individuals in the case of an intense TB epidemic occurrence (21).

In the present study, sensitivities of T-SPOT.TB test (73.0%) and NPV (64.3%) were found to be low. The T-SPOT.TB test might have false- negative results. However, T-SPOT.TB test was more successful than the QFT-G test in the control group.

A more recent study by Lee et al. comparing TST and 2 commercial kits utilizing IFN-γ measurements showed some sensitivity differences between these 2 IFN-γ based tests. However, when compared with TST, they were more successful in the detection of *M. tuberculosis* (11,22). Richeldi also demonstrated that tests based on IFN-γ in TB patients were more sensitive than TST (23). In that study, the sensitivities of T-SPOT.TB and QFT-G were higher than that of TST. In the same way, when the accepted TST cut-off value was ≥15 mm, the sensitivities of QFT-G and T-SPOT.TB tests among the participants were 42.2% and 51.3%, respectively (24).

Recently, studies have showed that IFN-γ based tests have higher sensitivities than TST-based tests (17,18,25). In the present study, sensitivities for QFT-G, T-SPOT.TB, and TST were detected as 78.4%, 73.0%, and 51.4%, respectively.

Mazurek et al. reported that there was no statistically significant difference between the specificities of TST and QFT-G (26). They speculated that the discordant results observed among the high-risk group for TB infection in the population may have been because of the lower TST specificity or lower QFT-G sensitivity. Moreover, they stated that additional studies may be needed if the results of TST and QFT-G tests are discordant in detecting TB risk.

Richeldi stated that blood tests are unlikely to yield a false-positive result in BCG-vaccinated people. The RD1 genomic region is present in few pathogenic NTM (e.g. *M. kansasii*, *M. marinum*, and *M. szulgai*), but is absent in *M. avium*. Tcell interferon-gamma release assays (TIGRAs) may therefore produce false-positive results in patients with some NTM infections although other clinical and laboratory features usually guide clinicians to the correct diagnosis in most such situations (27).

In conclusion, the present study demonstrated that NPV of both QFT-G and T- SPOT.TB tests were higher than that of TST for the diagnosis of active TB patients. The QFT-G and T-SPOT.TB tests were found superior to TST in routine clinical use because they are objective and display a superior performance for identifying active TB and LTBI among our populations with high rates of BCG vaccination. QFT-G test is easier to apply in the laboratory than T-SPOT.TB. However, large prospective longitudinal studies are needed to precisely identify the factors influencing the test performance and the results of TST (e.g., ≥15 mm, 15-20 mm, and ≥20 mm) are required to verify the effectiveness of QFT-G and T-SPOT.TB tests in LTBI diagnosis.

Acknowledgement

This study was presented as a poster at the ESM 2008 Congress, Panaiotov, Bulgaria. We would like to thank Kaan Medical and Ekin Medical for their technical assistance. We also would like to thank the volunteers who agreed to participate in the study. This study was financed by Refik Saydam National Hygiene Center, Ankara, Turkey.

References

1. www.who.int/vaccine_research/diseases/ari/en/index6.html
2. Gümüşlü F, Özkara Ş, Özkan S, Baykal F, Güllü Ü. The Report of tuberculosis in Turkey in 2007. Department of Tuberculosis Control, Ankara 2007.
3. Huebner RE, Schein MF, Bass JB. The tuberculin skin test. Clin Infect Dis 1993; 17: 968-75.
4. Fine PE, Bruce J, Ponnighaus JM, Nkhosa P, Harawa A, Vynnycky E. Tuberculin sensitivity: conversions and reversions in a rural African population. Int J Tubercl Lung Dis 1999; 3: 962-95.
5. Lee E, Holzman RS. Evolution and current use of the tuberculin test. Clin Infect Dis 2002; 34: 365-70.
6. Chadha VK. Tuberculin test. Indian J Pediatr 2001; 68: 53-8.
7. Fitzgerald D, Hass WD. Mycobacterium tuberculosis. In Mandell, JE., Bennett R. Dolin editors. Mandell, Bennett and Dolin's Principles and Practice of Infectious Diseases. 6th ed. Philadelphia: Churchill Livingstone; 2005. p.2852-2886.
8. Chapman AL, Munkanta M, Wilkinson KA, Pathan AA, Ewer K, Ayles H et al. Rapid detection of active and latent tuberculosis infection in HIV-positive individuals by enumeration of Mycobacterium tuberculosis-specific T cells. AIDS 2002; 16: 2285-93.
9. Dheda K, Lalvani A, Robert F. Performance of a T-cell-based diagnostic test for tuberculosis infection in HIV-infected individuals is independent of CD4 cell count. AIDS 2005; 19: 2031-41.
10. Dheda K, Udwadia ZF, Huggett JF, Johnson MA, Rook GA. Utility of the antigen-specific interferon-gamma assay for the management of tuberculosis. Curr Opin Pulm Med 2005; 11: 195-202.
11. Ferrara G, Losi M, Meacci M, Meccugni B, Piro R. Routine hospital use of a new commercial whole blood interferon-{gamma} assay for the diagnosis of tuberculosis infection. Am J Respir Crit Care Med 2005; 172: 631-5.

12. Lalvani A, Nagvenkar P, Udwadia Z, Pathan AA, Wilkinson KA. Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent Mycobacterium tuberculosis infection in healthy urban Indians. J Infect Dis 2001; 183: 469-77.

13. Scarpellini P, Tasca S, Galli L, Beretta A, Lazzarin A, Fortis C. Selected pool of peptides from ESAT-6 and CFP-10 proteins for detection of Mycobacterium tuberculosis infection. J Clin Microbiol 2004; 42: 3469-74.

14. Liebeschuetz S, Bamber S, Ewer K, Deeks J, Pathan AA, Lalvani A. Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. Lancet 2004; 364: 2196-2203.

15. Geiter LJ, Gordin FM, Hershfield E: Targeted tuberculin testing and treatment of latent tuberculosis infection. Am J Respir Crit Care Med 2000; 161: S221-S247.

16. Lee E, Holzman RS. Evolution and current use of the tuberculin test. Clin Infect Dis 2002; 34: 365-70.

17. Lalvani A, Pathan AA, McShane H, Wilkinson RJ, Latif M, Conlon CP, et al. Rapid detection of Mycobacterium tuberculosis infection by enumeration of antigen-specific T cells. Am J Respir Crit Care Med 2001; 163: 824-8.

18. Pai M, Riley LW, Colford JM. Interferon gamma assays in the immunodiagnosis of tuberculosis:a systematic review. Lancet Infect Dis 2004; 4: 761-76.

19. Mori T, Sakatani M, Yamagishi F, Takashima T, Kawabe Y, Nagao K et al. Specific detection of tuberculosis infection: an interferon-gamma based assay using new antigen. Am. J Respir Crit Care Med 2004; 170: 59-64.

20. Mazurek GH, LoBue PA, Daley CL, Bernardo J, Lardizabal AA. Comparison of a whole blood interferon gamma assay with tuberculin skin testing for detecting latent Mycobacterium tuberculosis infection. JAMA 2001; 286: 1740-47.

21. Miyashita H, Higuchi K, Higashiyama N, Oki S, Harada N. Detection of tuberculosis infection using a whole blood interferon gamma assay in a contact investigation–evaluation using QFT-G TB-2G. Kekkaku 2005; 80: 557-64.

22. Lee JY, Choi HJ, Park IN, Hong SB, Oh YM. Comparison of two commercial interferon-gamma assays for diagnosing Mycobacterium tuberculosis infection. Eur Respir J 2006; 28: 24-30.

23. Richeldi L: An update on the diagnosis of tuberculosis infection. Am J Respir Crit Care Med 2006; 174: 736-42.

24. Arend SM, Thijsen SF, Leyten EM, Bouwman JJ, Franken WP. Comparison of two interferon-gamma assays and tuberculin skin test for tracing tuberculosis contacts. Am J Respir Crit Care Med 2007; 175: 529-31.

25. American Thoracic Society. Targeted tuberculin testing and treatment of latent tuberculosis infection. Am J Respir Crit Care Med 2000; 161: 221-47.

26. Mazurek GH, Zajdowicz MJ, Hankinson AL, Costigan DJ, Toney SR. Detection of Mycobacterium tuberculosis infection in United States Navy recruits using the tuberculin skin test or whole-blood interferon-gamma release assays. Clin Infect Dis 2007; 45: 826-36.

27. http://www.ersbuyersguide.org/887-latest-issue.htm, "Blood tests for latent tubercolosis, L. Richeldi, 2007"