Le Infezioni in Medicina, n. 4, 525-531, 2024
doi: 10.53854/liim-3204-11

Performance of the T-SPOT.TB test in patients with indeterminate QuantiFERON-TB Gold Plus results: proposal for an algorithm for the diagnosis of Latent Tuberculosis Infection

Monica Pagnoncelli, Marco Arosio, Alessandro Genovesi,Gavino Napolitano, Claudio Farina
Clinical Microbiology and Virology Laboratory, Papa Giovanni XXIII Hospital, Bergamo, Italy

Article received 7 August 2024 and accepted 10 November 2024

SUMMARY

Latent Tuberculosis Infection (LTBI) is a state of persistent immune response to *Mycobacterium tuberculosis* complex antigens without clinical, radiological and microbiological signs of active disease. Effective diagnosis and preventive treatment of LTBI are crucial for tuberculosis (TB) control, especially in high-risk groups. Currently, two main tests are used for LTBI diagnosis: the Tuberculin Skin Test (TST) and the Interferon-Gamma Release Assays (IGRA), including the QuantiFERON-TB Gold Plus (QFT-Plus) and the T-SPOT.TB.

Our study evaluated the performance of the T-SPOT.TB test in patients with indeterminate QFT-Plus results, using data from the Clinical Microbiology and Virology Laboratory (M&V) of Papa Giovanni XXIII Hospital in Bergamo, Italy. Blood samples from patients tested for LTBI with QFT-Plus from January 1, 2017 to May 15, 2024 were analyzed. The QFT-Plus is the most widely used test in routine diagnostics for LTBI screening due to the availability of automated systems.

Out of 20,995 samples tested with QFT-Plus, 576 (2.7%) gave indeterminate results. In all cases of indeterminate QFT-Plus results, M&V recommends performing the T-SPOT.TB test. However, of the 576 patients who obtained an indeterminate outcome, only 137 (23.8%) followed the indication.

The T-SPOT.TB provided a definitive result in 87.6% of the cases, resolving 120 (80 negative and 40 positive) of 137 indeterminate QFT-Plus outcomes. Specifically, 78 of 92 cases, equal to 84.8%, were settled when the T-SPOT.TB test was performed within 30 days of the QFT-Plus.

The T-SPOT.TB test has shown potential effectiveness in addressing indeterminate QFT-Plus results (84.8% resolution), indicating its possible role as a complementary diagnostic tool for LTBI.

The proposed algorithm for LTBI screening is based on national and international guidelines recommending the use of the TST and/or an IGRA test for individuals at risk. However, it particularly emphasizes the use of QFT-Plus, due to its practicality and rapid execution, while recommending the addition of the T-SPOT.TB within 30 days in cases of indeterminate QFT-Plus results. Nevertheless, the conclusions should be regarded as preliminary and require confirmation through larger or controlled studies.

Keywords: Latent Tuberculosis Infection, T-SPOT.TB, QuantiFERON-TB Gold Plus, indeterminate results.

INTRODUCTION

Tuberculosis (TB), caused by microorganisms belonging to the *Mycobacterium tuberculosis* complex [1], has afflicted humanity since prehistoric times and continues to represent a global

Corresponding author
Monica Pagnoncelli
E-mail: monica.pagnoncelli@unimi.it

public health challenge. Latent Tuberculosis Infection (LTBI) is a state of persistent immune response to antigens of the *M. tuberculosis* complex without clinical, radiological and microbiological signs of active disease. About 95% of individuals infected with *M. tuberculosis* complex do not develop active disease because their immune system is able to contain the pathogenic action of the tubercle bacilli, some of which, while having a low replicative capacity, remain viable within macrophages in a quiescent state. In the remaining 5% of individuals, the infection progresses to active disease, with half of these cases occurring within two years. The probability of progression to active TB decreases over time, but remains a lifelong risk. This is particularly associated with weakened immune defenses, as in cases of immunosuppression or immunosuppressive therapy [2].

It is estimated that about a quarter of the world's population is affected by LTBI [3]. Although these individuals are not contagious and do not pose an immediate risk of disease transmission, they represent a significant source of potential TB cases. Therefore, the diagnosis of LTBI is a crucial component of TB control programs, and identification of individuals with LTBI is essential for the implementation of preventive chemotherapy with anti-tubercular drugs [4, 5].

Screening for LTBI is particularly focused on high-risk groups, such as people with HIV (PWH) and immunocompromised individuals. Other categories tested include close contacts of active TB cases, immigrants from high-incidence countries, Health Care Workers (HCW) and people with conditions that increase their risk of developing TB if they become infected, such as drug addicts, alcoholics and homeless [6-10].

Currently, two tests are available for the diagnosis of LTBI: the Tuberculin Skin Test (TST) and the Interferon-Gamma Release Assays (IGRA), specifically the QuantiFERON®-TB Gold Plus (QFT®-Plus; Qiagen, Hilden, Germany) and the T-SPOT®.TB (Oxford Immunotec, Abingdon, UK) [11].

The TST (or Mantoux test) measures an *in vivo* delayed hypersensitivity reaction to the intradermal injection of purified protein derivative (PPD), a cocktail of antigens extracted from the *M. tuberculosis* complex, some of which are common to various Non-Tuberculous Mycobacteria (NTM) and the Bacille Calmette-Guérin (BCG) vaccine strain.

IGRA tests are *in vitro* immunological assays that detect the immune response to specific peptide antigens that simulate mycobacterial proteins, particularly ESAT-6 (Early Secretory Antigen Target-6) and CFP-10 (Culture Filtrate Protein-10), encoded by genes within the Region of Difference-1 (RD-1) of the *M. tuberculosis* complex genome. RD-1 is present in all species of the complex, except the BCG vaccine strain, and is absent in most NTM, excluding *M. kansasii*, *M. marinum*, *M. szulgai* and *M. riyadhense*. Therefore, the specificity of IGRA tests is high, as results are not affected by previous BCG vaccination and most NTM infections, unlike the TST [12, 13].

The World Health Organization (WHO), as well as the Region of Lombardy (Italy) and other national and international guidelines, recommends a diagnostic-therapeutic algorithm that includes the TST or an IGRA test to screen for LTBI in high-risk categories. Individuals in these groups with a positive LTBI test are candidates for treatment, but must undergo thorough evaluation to exclude active disease, through clinical examination and chest radiography. If signs, symptoms, or any radiological abnormalities are present, further diagnostic investigations for TB and other pathologies are required. If active TB is ruled out, these individuals can start LTBI treatment [6-10].

The QFT-Plus test is generally the most widely used in routine diagnostics for LTBI screening, due to the availability of automated systems but, in cases of indeterminate QFT-Plus results, Clinical Microbiology and Virology Laboratory (M&V) of Papa Giovanni XXIII Hospital in Bergamo suggests performing the T-SPOT.TB test.

The aim of this study is to share our findings regarding the performance evaluation of the T-SPOT.TB method in patients with indeterminate QFT-Plus results, in order to enable the diagnosis of LTBI even in these cases and propose a new diagnostic algorithm.

MATERIALS AND METHODS

Tested samples

Data for this study were obtained from the computer archive of M&V and extracted using *Virtuoso Plus* (Dedalus SpA, Milan, Italy) software, which contains personal and clinical-epidemiological references related to the samples received. Specifically, blood samples tested for LTBI with QFT-Plus from January 1, 2017 to May 15, 2024, from

hospitalized patients and individuals attending the Blood Collection Center were considered.

QuantiFERON-TB Gold Plus (QFT-Plus)

The QFT-Plus test evaluates cell-mediated immune responses to peptide antigens that simulate mycobacterial proteins. In fact, T lymphocytes in heparinized whole blood from individuals sensitized *in vivo* to tuberculosis antigens respond with measurable interferon-gamma (IFN-γ) production after new *in vitro* antigenic stimulation. IFN-γ is detected and quantified (IU/mL) through an Enzyme-Linked Immunosorbent Assay (ELISA).

The QFT-Plus kit includes a Nil Tube (negative control), a Mitogen Tube (positive control) and two TB Antigen Tubes, TB Antigen Tube 1 (TB1) and TB Antigen Tube 2 (TB2). Both tubes contain a peptide cocktail simulating ESAT-6 and CFP-10, in order to stimulate cell-mediated immune responses from CD4+ T-helper lymphocytes. The TB2 tube contains an additional set of peptides designed to elicit responses from CD8+ cytotoxic T lymphocytes.

A QFT-Plus assay is considered positive for an IFN-γ response to either TB Antigen Tube that is significantly above the negative control value. The results must be interpreted considering that the IFN-γ level of the Nil Tube is subtracted from the IFN-γ amount of the TB Antigen Tubes and the Mitogen Tube.

According to the commercial kit, the threshold value between positive and negative result is 0.35 IU/mL (≥0.35 IU/mL: positive; <0.35 IU/mL: negative). However, for the first sample received, it was decided to introduce a "gray area" in the results interpretation, although universally accepted reference values are still lacking. M&V identifies these values in the range of >0.30 to ≤1 IU/mL, where the outcome is considered doubtful, as no definitive interpretation is possible.

For AMCLI (Associazione Microbiologi Clinici Italiani), the "gray area" falls within the values 0.20 and 0.70 [14]; however, our laboratory decided to set the lower threshold value at 0.30 as we frequently observed that values between 0.20 and 0.30, when re-evaluated in subsequent tests, remained consistently negative. We set the upper threshold at ≤1, as we observed that values between 0.70 and 1 tended to become negative upon retesting in approximately 10% of cases.

Thus, for the first sample, the test is considered positive if the value of either TB Antigen Tube is >1 IU/mL and negative if it is ≤0.30 IU/mL, while, in case of doubtful result (range between >0.30 and ≤1 IU/mL), the submission of a second sample after 30 days is required, which instead follows the criteria proposed by the kit.

An indeterminate result is obtained in cases of low response to the Mitogen (<0.5 IU/mL) and no reactivity to specific antigens. This may be due to an insufficient number of lymphocytes, their inability to secrete IFN-γ or a reduction in their activity as a consequence of improper sample handling.

T-SPOT.TB

The T-SPOT.TB test is an *in vitro* diagnostic test that detects T lymphocytes producing IFN-γ in response to stimulation with peptides simulating ESAT-6 and CFP-10 antigens of the *M. tuberculosis* complex. The T-SPOT.TB uses a simplified version of the enzyme-linked immunospot (ELISPOT) methodology to enumerate activated T cells.

The test is performed on whole blood samples, from which peripheral blood mononuclear cells (PBMCs) are separated, washed and counted to ensure that an adequate number of cells is used, even for samples with a low number of cells due to a weakened immune system (immunocompromised and immunosuppressed subjects). PBMCs, placed into the wells of a microtiter plate, are exposed to a positive control containing phytohemagglutinin (a mitogenic stimulator indicating cell functionality), a negative control (Nil) and two separate panels of *M. tuberculosis* complex specific antigens (Panel A containing ESAT-6 and Panel B containing CFP-10).

After a first incubation at 37°C for 16-20 hours, any T lymphocytes previously sensitized *in vivo* to mycobacterial antigens release IFN-γ, which is captured by specific anti-IFN-γ antibodies coating the wells. The cells and other unbound materials are removed by washing and a second incubation is carried out with anti-IFN-γ antibodies conjugated to alkaline phosphatase, directed against a different epitope of the cytokine. The subsequent addition of a substrate leads to the formation of insoluble precipitate spots at the bottom of the wells. Each spot corresponds to a reactive cell; thus, the count of the spots indicates the number of activated T lymphocytes.

The results are interpreted by subtracting the spot count in the negative control well from the spot

count in each of the Panels. According to the commercial kit the test is positive if the number of spots is ≥6 (Panel A-Nil and/or Panel B-Nil) and negative if the number of spots is ≤5 (both Panel A-Nil and Panel B-Nil). For the first sample received M&V considers the test positive if the spot count is ≥ 8, negative if it is ≤4 and doubtful ("gray area") if it is within the range >4 and <8. In the latter case the submission of a second sample after 30 days is required, which instead follows the criteria proposed by the kit.
An indeterminate result may be obtained if the sample has a low number of PBMCs, contains interfering substances or in case of a high background of IFN-γ (Nil>10 spots).

RESULTS

From January 1, 2017 to May 15, 2024, a total of 20,995 samples were tested for LTBI through QFT-Plus at M&V, with the following results: 18,279 (87.1%) negative, 1,338 (6.4%) positive, 802 (3.8%) doubtful and 576 (2.7%) indeterminate (Figure 1).
In all cases of indeterminate QFT-Plus results, M&V recommends performing the T-SPOT.TB test. However, of the 576 patients who obtained an indeterminate outcome, only 137 (23.8%) followed the indication. Specifically, 92 underwent the T-SPOT.TB within 30 days, 23 between 31 and 60 days and 22 over 60 days after the QFT-Plus. Among the 92 samples of patients tested within 30 days of the QFT-Plus, 84.8% (78 samples) yielded a definitive result (51 negative and 27 positive), while 15.2% (14 samples) remained doubtful or indeterminate. For the 23 samples analyzed with the T-SPOT.TB 31 to 60 days after the QFT-Plus, the diagnosis was possible in 91.3% of cases (21 samples: 12 negative and 9 positive). The 22 samples assayed beyond 60 days after the first IGRA test produced a definitive result in 95.5% of cases (21 samples: 17 negative and 4 positive) (Table 1).
Overall, the introduction of the T-SPOT.TB resolved 120 (87.6%) of 137 indeterminate cases, resulting in 80 (58.4%) negative, 40 (29.2%) positive, 7 (5.1%) doubtful and 10 (7.3%) indeterminate (Figure 2).

DISCUSSION

In the management of TB, it is crucial to distinguish between latent infection and active disease [15].
The diagnosis of LTBI is based on the detection of an immune response to the *M. tuberculosis* complex, whereas the diagnosis of active disease fo-

Figure 1
QFT-Plus results for the 20995 samples tested from January 1, 2017 to May 15, 2024.

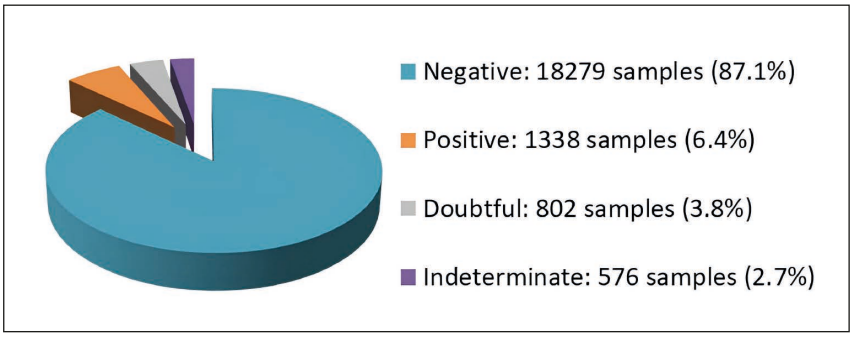

Table 1 - T-SPOT.TB results for patients with indeterminate QFT-Plus outcome, categorized by the time interval between the two tests.

T-SPOT.TB results	*≤30 days*	*31-60 days*	*>60 days*	*Total*
Negative	51	12	17	80
Positive	27	9	4	40
Doubtful	5	1	1	7
Indeterminate	9	1	0	10
Total	92	23	22	137

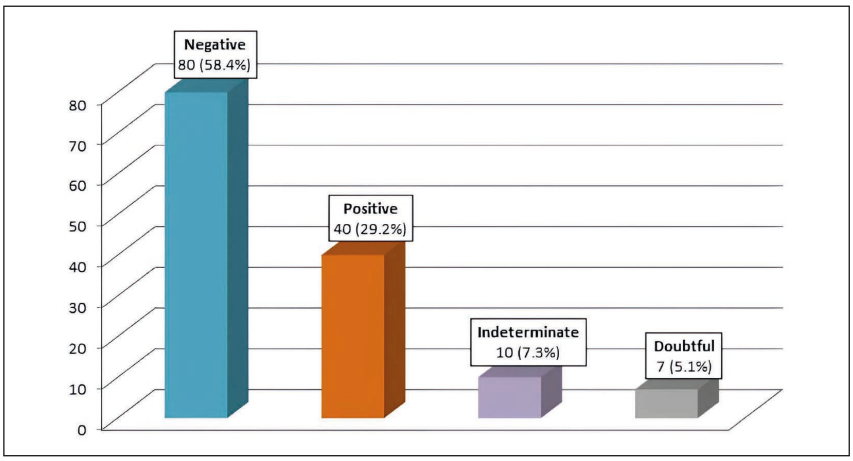

Figure 2
T-SPOT.TB results for patients with indeterminate QFT-Plus outcome.

cuses on identifying the bacillus in biological samples [16]. Two tests are employed for the diagnosis of LTBI: the Tuberculin Skin Test (TST) and the Interferon-Gamma Release Assays (IGRA).
Although TST is cost-effective and relatively simple to administer, it has notable limitations, including false positives in individuals vaccinated with BCG or infected with NTM and false negatives due to cellular immunity deficits or insufficient time since exposure.
IGRA tests have higher specificity than TST, especially in those vaccinated with BCG, individuals exposed to NTM and immunosuppressed subjects. The presence of a positive control (Mitogen) allows for the identification of anergic subjects, in whom a negative test result is actually to be considered indeterminate, as it may be due to an insufficient immune response. Furthermore, as laboratory tests, IGRA are reproducible, not operator-dependent, and not subject to the booster effect [17].
For these reasons, they are increasingly recommended, especially in low-incidence countries but the use of the TST is still favored in high-incidence areas with limited resources due to its low cost [18]. However, both the TST and IGRA tests cannot differentiate between active and latent TB, nor distinguish between reactivation and reinfection. Therefore, the patient's clinical history and epidemiological context should always be taken into account when interpreting test results [19].
The diagnostic-therapeutic algorithm proposed by the Region of Lombardy, in line with what is suggested by international guidelines, recommends the execution of the TST or an IGRA test in at-risk subjects. The tests can also be performed sequentially to increase specificity (for example, in the case of previous BCG vaccination) or sensitivity (for example, in immunocompromised subjects) [6-10].
In routine diagnostics of M&V, QFT-Plus is used for LTBI screening, but it has been found that during the period from January 1, 2017 to May 15, 2024, this test yielded an indeterminate result for 576 patients, equal to 2.7% of the total. The causes can be various and are not always due to the methods of sample collection, storage and processing (operator-dependent procedures), but are sometimes related to the presence of interfering substances, such as in autoimmune diseases, or the administration of immunosuppressive drugs that alter cell-mediated responses [20].
In cases of indeterminate QFT-Plus results, a note is included in the patient's medical report recommending the execution of the T-SPOT.TB test. Of the 576 patients who obtained an indeterminate outcome, 137 (23.8%) followed this indication.
In particular, 78 out of 92 cases, equal to 84.8%, were settled when the T-SPOT.TB test was performed within 30 days of the QFT-Plus. From a previous evaluation (unpublished data), we found that 90% of indeterminate QFT-Plus results were confirmed when retested by the same method within the month; therefore, our recommendation is to perform T-SPOT.TB in these cases, whereas, beyond the month, it may be useful to simply repeat the QFT-Plus.
Doubtful results were not considered in our study

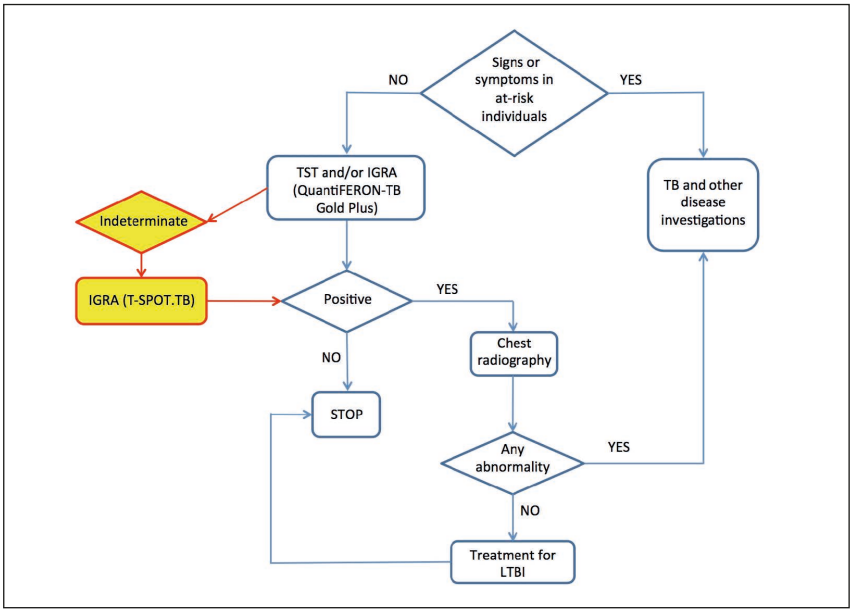

Figure 3
Proposal for a new algorithm for LTBI diagnosis (Region of Lombardy protocol modified by M&V).

because a previous assessment conducted by our laboratory (unpublished data) showed that it was possible to obtain definitive outcomes (either positive or negative) by repeating the QFT-Plus.
The ability of the T-SPOT.TB to resolve indeterminate outcomes may be due to improper sample handling for the QFT-Plus or better performance of the T-SPOT.TB in these cases. Although this test is more laborious and complex to perform as it is not automated, it seems to be useful in resolving indeterminate cases of QFT-Plus, allowing a diagnosis to be reached even in these instances [21, 22].

CONCLUSIONS

The T-SPOT.TB test has shown potential efficacy in addressing indeterminate QFT-Plus results (84.8% resolution), indicating its possible role as a complementary diagnostic tool for LTBI.
The proposed algorithm for LTBI screening is based on national and international guidelines, which recommend performing the TST and/or an IGRA test on at-risk individuals. However, it particularly emphasizes the use of QFT-Plus due to its practicality and rapid execution, while recommending the addition of the T-SPOT.TB within 30 days in cases of indeterminate QFT-Plus results (Figure 3).
Nevertheless, these findings should be considered preliminary and require confirmation through larger or controlled studies. Although the overall patient population tested with QFT-Plus test was substantial, the number of cases tested with the T-SPOT.TB was limited, as not all patients with indeterminate QFT-Plus results underwent T-SPOT.TB. Furthermore, the indeterminate results have not been retested with the QFT-Plus, making these data potentially valuable for future larger studies.

Conflict of interest
None.

Funding
This research did not receive any specific grant from public, commercial or not-for-profit agencies.

REFERENCES

[1] Azé J, Sola C, Zhang J, et al. Genomics and Machine Learning for Taxonomy Consensus: The Mycobacterium tuberculosis Complex Paradigm. *PLoS ONE*. 2015; 10(7).
[2] Boom WH, Schaible UE, Achkar JM. The knowns and unknowns of latent Mycobacterium tuberculosis infection. *J Clin Invest*. 2021; 131(3).
[3] World Health Organization. Global tuberculosis report 2023. Geneva, Switzerland; 2023. Available at: https://www.who.int/teams/global-tuberculosis-pro-

gramme/tb-reports/global-tuberculosis-report-2023. Accessed April 6, 2024.
[4] World Health Organization. The End TB Strategy. Geneva, Switzerland; 2015. Available at: https://www.who.int/teams/global-tuberculosis-programme/the-end-tb-strategy. Accessed March 28, 2024.
[5] Centers for Disease Control and Prevention. Guidelines for the Treatment of Latent Tuberculosis Infection: Recommendations from the National Tuberculosis Controllers Association and CDC. Atlanta, USA; 2020. Available at: https://www.cdc.gov/mmwr/volumes/69/rr/pdfs/rr6901a1-H.pdf. Accessed May 8, 2024.
[6] World Health Organization. Latent tuberculosis infection: updated and consolidated guidelines for programmatic management. Geneva, Switzerland; 2018. Available at: https://www.who.int/publications/i/item/9789241550239. Accessed March 20, 2024.
[7] European Centre for Disease Prevention and Control. Programmatic management of latent tuberculosis infection in the European Union. Stockholm, Sweden; 2018. Available at: https://www.ecdc.europa.eu/en/publications-data/programmatic-management-latent-tuberculosis-infection-european-union. Accessed March 20, 2024.
[8] Bollettino Ufficiale Regione Lombardia. Revisione ed aggiornamento degli interventi di sorveglianza, prevenzione, profilassi e controllo della tubercolosi in Regione Lombardia. Serie Ordinaria n. 29 - Martedì 18 luglio 2017.
[9] National Institute for Health and Clinical Excellence. Tuberculosis. Clinical diagnosis and management of tuberculosis, and measures for its prevention and control. London, UK; 2011. Available at: https://www.bsuh.nhs.uk/library/wp-content/uploads/sites/8/2020/06/NICE-tuberculosis.pdf. Accessed April 7, 2024.
[10] Associazione Microbiologi Clinici Italiani. Percorso diagnostico. Diagnosi immunologica dell'infezione tubercolare. Milan, Italy; 2023. Available at: https://www.amcli.it/wp-content/uploads/2024/02/2023-19_DIAGNOSI-IMMUNOLOGICA-DELLINFEZIONE-TUBERCOLARE.pdf. Accessed April 3, 2024.
[11] Zellweger JP, Sotgiu G, Corradi M, et al. The diagnosis of latent tuberculosis infection (LTBI): currently available tests, future developments, and perspectives to eliminate tuberculosis (TB). *Med Lav*. 2020; 111(3): 170-183.
[12] Word Health Organization. Use of alternative interferon-gamma release assays for the diagnosis of TB infection: WHO policy statement. Geneva, Switzerland; 2022. Available at: https://www.who.int/publications/i/item/9789240042346. Accessed April 5, 2024.
[13] European Centre for Disease Prevention and Control. Use of interferon-gamma release assays in support of TB diagnosis. Stockholm, Sweden; 2011. Available at: https://www.ecdc.europa.eu/en/publications-data/public-health-guidance-use-interferon-gamma-release-assays-support-tb-diagnosis. Accessed April 5, 2024.
[14] Associazione Microbiologi Clinici Italiani. Refertazione Quantiferon-Plus: considerazioni emerse durante la tavola rotonda "Nuovi approcci clinici, microbiologici e diagnostici alla TB latente" – XLVII Congresso AMCLI. A cura del GLaMic, Gruppo di Lavoro Micobatteri. Milan, Italy; 2019. Available at: https://www.amcli.it/wp-content/uploads/2015/09/Consensus_QUANTIFERON_versione-del-2apr2019.pdf. Accessed October 10, 2024.
[15] Gong W, Wu X. Differential Diagnosis of Latent Tuberculosis Infection and Active Tuberculosis: A Key to a Successful Tuberculosis Control Strategy. *Front Microbiol*. 2021; 12: 745592.
[16] Associazione Microbiologi Clinici Italiani. Percorso diagnostico. Tubercolosi. Milan, Italy; 2023. Available at: https://www.amcli.it/wp-content/uploads/2024/02/2023-18_TUBERCOLOSI-E-ALGORITMO.pdf. Accessed April 3, 2024.
[17] Zhang Y, Zhou G, Shi W, et al. Comparing the diagnostic performance of QuantiFERON-TB Gold Plus with QFT-GIT, T-SPOT.TB and TST: a systematic review and meta-analysis. *BMC Infect Dis*. 2023; 23(1): 40.
[18] Heireman L, Bruynseels P, Camps K, et al. Comparison of the QuantiFERON-TB® Gold Plus on LIAISON® XL and T-SPOT.TB for the diagnosis of latent Mycobacterium tuberculosis infection in a low tuberculosis incidence population. *Diagn Microbiol Infect Dis*. 2022; 102(3): 115613.
[19] Goletti D, Delogu G, Matteelli A, et al. The role of IGRA in the diagnosis of tuberculosis infection, differentiating from active tuberculosis, and decision making for initiating treatment or preventive therapy of tuberculosis infection. *Int J Infect Dis*. 2022; 124 (Suppl. 1): S12-S19.
[20] Zhou G, Luo Q, Luo S, et al. Indeterminate results of interferon gamma release assays in the screening of latent tuberculosis infection: a systematic review and meta-analysis. *Front Immunol*. 2023; 14: 1170579.
[21] Zhang L, Zhang J, Yang S, et al. Performance of LTBI Screening in Patients with Rheumatic Diseases Using Two Different Interferon- Gamma Releasing Assays. *Front Biosci* (Landmark Ed). 2022; 27(10): 282.
[22] Fernández-Blázquez A, Argüelles Menéndez P, Sabater-Cabrera C, et al. Diagnosis of Tuberculous Infection in Immunosuppressed Patients and/or Candidates for Biologics Using a Combination of 2 IGRA Tests: T-SPOT.TB/QuantiFERON TB Gold In-Tube vs. T-SPOT.TB/QuantiFERON TB Gold Plus. *Arch Bronconeumol*. 2022; 58(4): 305-310.