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CORRELATION BETWEEN MULTIPLEX PCR (GENEXPERT MTB/RIF) AND THE QUANTIFERON-TB GOLD PLUS ASSAY IN THE DIAGNOSIS OF ACTIVE TUBERCULOSIS

Youssef Bighouab*, Hamza Bougoun, Rime Messaoudi, Bernoussi Taha, Benouda Amina

Department of Medical Microbiology, Cheikh Zaid Hospital, Rabat, Morocco.

***Corresponding Author: Youssef Bighouab**

Department of Medical Microbiology, Cheikh Zaid Hospital, Rabat, Morocco.

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ABSTRACT

Provide on a separate page an abstract of not more than 150-250 words. **Background:** The control of *Mycobacterium tuberculosis* infection relies on the complementarity of microbiological and immunological tools. This study evaluates the diagnostic correlation between the multiplex PCR GeneXpert MTB/RIF and the QuantiFERON-TB Gold Plus (QFT-Plus) assay in a hospital setting. **Methods:** A retrospective, analytical, and observational study was conducted at the microbiology laboratory of Cheikh Zaid Hospital in Rabat over a 6-year period (2021–2026). The concordance analysis included a cohort of 43 patients who underwent simultaneous testing with a positive QFT-Plus and a GeneXpert MTB/RIF assay. **Results:** The overall mean age was 50.2 years with a sex ratio of 0.95, and 83.8% of the tests were performed on respiratory specimens. The analytical cross-tabulation highlighted.

- 21% concordance (QFT+ / PCR+): Indicating active pulmonary tuberculosis, confirmed 100% on respiratory specimens.
- 79% discordance (QFT+ / PCR-): Reflecting a predominance of latent tuberculosis infections (LTBI) in our endemic context, primarily screened prior to immunosuppression.

In-depth analysis of the bacilliferous subgroup (QFT+ / PCR+) revealed:

- An immunological alert signature: A quantitative hypersecretion of interferon-gamma (≥ 4 IU/mL) and a biomarker inversion (TB2 > TB1), translating the activation of CD8+ cytotoxic T lymphocytes.
- A demographic shift: A clear rejuvenation (mean age of 36.3 years; 44.4% aged 20–29 years) and a strong masculinization (sex ratio of 2). Furthermore, no positive first-line PCR was subsequently followed by a QFT-Plus test.

Conclusion: These two tests are strictly complementary. The discovery of a high-risk QFT-Plus profile (≥ 4 IU/mL and TB2 > TB1) must immediately raise suspicion of an evolving lesion and trigger a GeneXpert test. Maximum diagnostic vigilance is required for respiratory specimens in young male subjects, as this population is at the highest risk for contagious and cavitary tuberculosis.

KEYWORDS: Tuberculosis, QuantiFERON-TB Gold Plus, GeneXpert MTB/RIF, Diagnostic Concordance, Multiplex PCR, Interferon-gamma.

INTRODUCTION

Tuberculosis (TB) remains one of the leading infectious causes of mortality worldwide despite considerable progress in diagnostics and treatment.[1] According to the World Health Organization (WHO), approximately 10.8 million new TB cases were recorded in 2023, resulting in over 1.2 million deaths.[1] The causative agent, *Mycobacterium tuberculosis*, continues to represent a major public health challenge, particularly in endemic areas and resource-limited countries.[1, 6] In Morocco, the incidence remains high, with pulmonary and extrapulmonary forms heavily impacting the healthcare system.[6]

Effective TB control relies on rapid and reliable detection.[1, 2] Conventional diagnostic methods exhibit

well-known limitations: microscopy lacks sensitivity in paucibacillary forms, while mycobacterial culture requires several weeks to yield definitive results.[13] The emergence of molecular biology has profoundly transformed this approach.[4] Multiplex PCR techniques, such as the GeneXpert MTB/RIF, allow for the direct and simultaneous detection of mycobacterial DNA and rifampicin resistance within hours, offering superior sensitivity and acting as a diagnostic gold standard for active disease.[4, 10]

Parallel to molecular advances, immunological tests assessing the host's specific cellular response have seen widespread adoption.[2, 13] Interferon-Gamma Release Assays (IGRAs), notably the QuantiFERON-TB Gold Plus (QFT-Plus), measure interferon-gamma (IFN-gamma) production by T lymphocytes stimulated with specific mycobacterial antigens (ESAT-6 and CFP-10).[13, 14] These assays offer superior specificity over the tuberculin skin test (TST), particularly in BCG-vaccinated populations, securing their role in screening for latent tuberculosis infection (LTBI).[2, 14]

However, both modalities possess inherent limitations.[3, 11] IGRAs cannot reliably distinguish between LTBI and active disease, while multiplex PCR solely detects the pathogen's genetic material and may miss latent states.[3, 4, 11]

The objective of this study was to evaluate the diagnostic correlation between multiplex PCR (GeneXpert MTB/RIF) and the QFT-Plus test to appreciate their respective performances, complementarity, and role in managing patients suspected of infection.

MATERIALS AND METHODS

study design and setting This was a retrospective, analytical, observational study conducted at the Department of Medical Microbiology of Cheikh Zaid Hospital, a tertiary referral university hospital in Rabat, Morocco. The study covered a 6-year period from January 2021 to May 2026.

study population and selection criteria The study investigated clinical records of patients who underwent diagnostic testing for *Mycobacterium tuberculosis* infection.

- Inclusion Criteria: Patients who had a positive QuantiFERON-TB Gold Plus test during the study period and who also underwent concurrent mycobacterial DNA detection via GeneXpert MTB/RIF, with complete and validated laboratory records.
- Exclusion Criteria: Patient files with incomplete clinical or biological data, or those presenting indeterminate QFT-Plus results or invalid/error codes on GeneXpert without a valid repeat test.

A total of 109 patients tested positive for QFT-Plus during the study period. Among them, a cohort of 43 patients who underwent simultaneous testing with both QFT-Plus and GeneXpert MTB/RIF was isolated for analytical correlation.

laboratory procedures

1. quantiferon-tb gold plus (qft-plus) assay The QFT-Plus assay (QIAGEN®, Hilden, Germany) was performed according to the manufacturer's guidelines. Whole blood was collected in four specific tubes: Nil, TB1, TB2, and Mitogen. Following incubation at 37°C for 16–24 hours, IFN-gamma concentrations were quantified using an ELISA method. A test was deemed positive when the antigenic response (TB1-Nil and/or TB2-Nil) reached the threshold of ≥ 0.35 IU/mL.

2. multiplex real-time pcr (genexpert mtb/rif) Molecular detection was performed using the automated GeneXpert MTB/RIF system. This real-time multiplex PCR detects the DNA of the *M. tuberculosis* complex alongside mutations in the rpoB gene. Analyses were conducted on varied clinical specimens (expectorations, bronchial aspirates, cerebrospinal fluid, biopsies, and lymph node aspirates).

3. data collection and statistical analysis Data collection and analysis were conducted using Microsoft Excel. Quantitative variables were expressed as means ± standard deviations, while qualitative variables were reported as absolute frequencies and percentages.

RESULTS AND DISCUSSION

global quantitative immune responses Over the study period, 43 patients underwent joint testing with a positive QFT-Plus and a GeneXpert PCR. The mean quantitative immunological response within this cohort was 3.17 IU/mL for TB1-Nil and 2.85 IU/mL for TB2-Nil. The joint evaluation of the QFT-Plus and GeneXpert MTB/RIF assays immediately reveals a highly reactive cell-mediated immune response, with mean IFN-gamma levels vastly exceeding the 0.35 IU/mL threshold.[1] However, IGRA pathophysiology evaluates immune memory, not direct pathogen detection.[2] These high serum concentrations alone cannot confirm active replication.[2, 3] The GeneXpert system bridges this gap by exploring the direct presence of mycobacterial DNA in situ,[4] formally distinguishing LTBI from active disease[1, 4] and allowing us to explore the predictive value of quantitative QFT-Plus variations.

demographic characteristics of the co-tested cohort The mean age was 50.2 years (range: 1 to 88 years). The distribution exhibited a bimodal pattern: the largest group consisted of elderly patients aged 70–79 years (34.9%), followed by young adults aged 20–29 years (20.9%).

Table 1: Age distribution of the co-tested patient cohort.

Age Bracket (Years)	Number of Cases
0-9	2
10-19	4
20-29	9
30-39	3
40-49	3
50-59	3
60-69	3
70-79	15
≥ 80	1

The mean age of 50.2 years illustrates the current epidemiological dynamics in hospital settings. The bimodal distribution reflects two distinct realities: the peak among patients aged 70–79 years (34.9%) translates to the accumulation of lifetime exposure risks,[5] coupled with increased screening prior to immunosuppressive therapies in geriatrics.[3, 5] Conversely, the peak among young adults aged 20–29 years (20.9%) targets direct human-to-human transmission, where social interactions favor active bacillary circulation.[4, 6] The cohort demonstrated a balanced sex distribution, with 22 females (51.2%) and 21 males (48.8%), yielding a sex ratio of 0.95. This near-perfect parity contrasts with global active TB statistics, which consistently report a clear male predominance (ratio 1.5 to 2.0).[6] This is explained by an indication bias in hospital settings: LTBI screening heavily targets patients monitored for chronic inflammatory and rheumatological diseases, which predominantly affect females.[8]

temporal trends and distribution by clinical specimen nature The annual volume of simultaneous QFT-Plus and GeneXpert prescriptions exhibited a sharp upward trajectory over the 6-year period. Prescriptions were modest in 2022 (n=6) and 2023 (n=3), but significantly increased in 2024 (n=9), culminating in a peak of 22 simultaneous prescriptions in 2025. The marked rise in joint prescriptions aligns with documented post-pandemic epidemiological contexts,[9] illustrating a major diagnostic catch-up and clinical reactivation of latent infections due to delayed care.[6, 9]

fig. 1. annual evolution of simultaneous prescriptions for quantiferon-tb gold plus and genexpert mtb/rif assays.

Analysis of the anatomical origins of specimens subjected to GeneXpert MTB/RIF testing revealed a heavy predominance of respiratory tract matrices (83.8% of total tests). Expectoration (sputum) was the primary specimen tested (51.2%, n=22), followed by bronchial aspirates (32.6%, n=14). Extrapulmonary specimens comprised 16.2% of the cohort.

Table 2: Distribution of clinical specimens analyzed by GeneXpert MTB/RIF.

Specimen Nature	Number of Cases (n)	Percentage (%)
Sputum (Expectorations)	22	51.2%
Bronchial Aspirates	14	32.6%
Lymph Node Biopsy / Pus	3	7.0%
Cerebrospinal Fluid (CSF)	2	4.6%
Other (Pleural Fluid, Gastric Lavage)	2	4.6%

The predominance of the respiratory tract (83.8%) conforms to the natural history of the infection.[6, 10] GeneXpert yields optimal results here, with >90% sensitivity on sputum.[4, 10] Conversely, the extrapulmonary samples represent paucibacillary pathologies, requiring clinicians to exploit the complementarity of tests due to lower PCR sensitivity but near 100% specificity.[11, 12]

concordance and discordance analysis Cross-analysis revealed a diagnostic concordance (QFT+ / PCR+) in 21% of patients (n = 9), representing double diagnostic confirmation. Conversely, diagnostic discordance (QFT+ / PCR-) was observed in 79% of patients (n = 34).

Table 3: Diagnostic concordance and discordance between QuantiFERON-TB Gold Plus and GeneXpert MTB/RIF.

Cross-Result Profile	Number of Patients (n)	Percentage (%)
QFT Positive (+) and PCR Positive (+)	9	21%
QFT Positive (+) and PCR Negative (-)	34	79%

Quantitative evaluation of IFN-gamma concentrations demonstrated notably higher levels in the concordant group compared to the discordant group.

Table 4: Comparison of mean interferon-gamma concentrations (IU/mL) based on GeneXpert MTB/RIF results.

QuantiFERON-TB Gold Plus Parameter	Concordant Group (QFT+ / PCR+)	Discordant Group (QFT+ / PCR-)
Nil Tube	0.814 ± 1.183 IU/mL	0.464 ± 0.401 IU/mL
TB1 Tube	4.034 ± 4.084 IU/mL	2.943 ± 1.837 IU/mL
TB2 Tube	4.853 ± 2.571 IU/mL	2.870 ± 2.028 IU/mL
Mitogen	8.89 ± 3.15 IU/mL	6.00 ± 4.47 IU/mL

characteristics of the double-positive patients (concordant group) Isolation of the 9 patients with dual positivity highlighted a drastic demographic and clinical shift.

1. Age Shift: The mean age plummeted to 36.3 years, largely dominated by young adults aged 20–29 years (44.4% of confirmed active cases). This rejuvenation perfectly illustrates the classic epidemiological terrain of contagious, excretory pulmonary TB.[6, 10]
2. Sex Ratio Inversion: A distinct male predominance was recorded (6 men, 66.7%; 3 women, 33.3%), establishing a sex ratio of 2. This radical inversion matches WHO statistics for bacteriologically confirmed forms, driven by immunosuppressive and bronchial irritant cofactors.[6, 10]
3. Specimen Exclusivity: 100% of the *M. tuberculosis* molecular confirmations in this group were obtained strictly from respiratory specimens.

Table 5. Demographic and clinical characteristics of patients with diagnostic concordance (Double Positive: QFT+ / PCR+).

Characteristic	Number of patients (n = 9)	Percentage (%)
Sex		
Male	6	66.7%
Female	3	33.3%
Age Bracket (years)		
10 – 20	1	11.1%
20 – 29	4	44.4%
50 – 59	1	11.1%
≥ 60	3	33.3%
Origin of Positive PCR Specimen		
Expectorations (Sputum)	6	66.7%
Bronchial aspirates	3	33.3%
Extrapulmonary specimens	0	0.0%

clinical significance of concordance and alert thresholds The confrontation of our two diagnostic approaches reveals a concordance rate (QFT+ / PCR+) of 21%, alongside a major discordance rate (QFT+ / PCR-) of 79%. Far from reflecting an analytical limitation, this distribution illustrates the perfect immunopathological separation between quiescent LTBI and active, bacilliferous disease. The marked predominance of discordant profiles (79%) is fundamentally explained by the epidemiological reality of Morocco, where repeated community exposure generates a very high baseline LTBI prevalence.[5, 6] This finding aligns perfectly with meta-analyses confirming that while IGRA sensitivity exceeds 90%, its specificity for discriminating active disease from simple latency collapses in endemic zones.[14, 19]

quantitative predictive value of ifn-gamma: the 4 iu/ml alert threshold Beyond binary interpretation, quantitative analysis of IFN-gamma concentrations demonstrates that cytokine secretion intensity is intimately linked to disease activity.[13] In our series, bacilliferous patients (PCR+) displayed exceptionally high mean concentrations, approaching or exceeding 4 IU/mL. Conversely, discordant latent patients maintained significantly lower levels (under 3 IU/mL). This elevation of the immunological baseline is explained by continuous community circulation of *M. tuberculosis*, which leads to repeated antigenic re-exposures.[5] This constant booster effect maintains highly sensitized memory T cells in latent carriers, structurally elevating the IFN-gamma background noise.[5, 13] To overcome intense background LTBI immunity in endemic zones, our work demonstrates the necessity of raising the clinical alert threshold to ≥ 4 IU/mL.[13, 17]

the differential ratio tb2 > tb1: a cytotoxic signature More remarkably, this quantitative elevation is accompanied by a decisive biomarker inversion in the bacilliferous group: the IFN-gamma concentration in tube TB2 systematically exceeds that of tube TB1 (4.85 vs. 4.03 IU/mL). This empirical observation directly aligns with recent advances in mycobacterial immunology.[15, 18] While TB1 targets strictly CD4+

helper T cells,[14] TB2 incorporates optimized short peptides to simultaneously induce responses from both CD4+ and CD8+ cytotoxic T cells.[13, 14] The predominance of TB2 over TB1 in our confirmed patients validates this differential as an indirect signature of a CD8+ cytotoxic response triggered by active bacillary replication.[13]

study limitations Our study has several limitations. The retrospective design and single-center hospital setting may introduce selection bias. Additionally, the sample size of the co-tested cohort (n=43), while sufficient to establish statistically significant divergence, warrants expansion in larger multicenter prospective cohorts.

CONCLUSION

In conclusion, our study demonstrates that the QuantiFERON-TB Gold Plus (QFT-Plus) and GeneXpert MTB/RIF assays are strictly complementary, not interchangeable. The 21% concordance rate (QFT+ / PCR+) accurately identifies active pulmonary tuberculosis, while the 79% discordance (QFT+ / PCR-) reflects the high background prevalence of latent tuberculosis infection (LTBI) in our endemic Moroccan context.

Crucially, our findings provide two major diagnostic alerts for hospital practice.

1. An immunological signature for active disease: In endemic zones, a highly reactive QFT-Plus result (≥ 4 IU/mL) with a biomarker inversion (TB2 > TB1) must no longer be interpreted as simple latency. It indicates active CD8+ T-cell mobilization driven by bacillary multiplication.
2. A demographic shift: Active contagious forms drastically concentrate in young adults (mean age 36.3 years), with a strong male predominance (sex ratio of 2.0).

Therefore, the discovery of a high-risk QFT-Plus profile (≥ 4 IU/mL and TB2 > TB1) must immediately trigger a GeneXpert MTB/RIF assay. Maximum diagnostic vigilance regarding respiratory specimens is essential in young male subjects, as they carry the highest risk for contagious, cavitary TB. Integrating these distinct tools within a multidisciplinary algorithm remains the most efficient strategy for optimizing patient management.

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REFERENCES

1. World Health Organization. (Global Tuberculosis Report 2024). Geneva; World Health Organization: 2024.
2. Pai M, Denkinger CM, Kik SV, et al. (Gamma interferon release assays for detection of Mycobacterium tuberculosis infection). Clin Microbiol Rev, 2014; 27(1): 3-20.
3. Lewinsohn DM, Leonard MK, LoBue PA, et al. (Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children). Clin Infect Dis, 2017; 64(2): e1-e33.
4. Boehme CC, Nicol MP, Nabeta P, et al. (Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study). Lancet, 2011; 377(9776): 1495-1505.
5. Houben RMGJ, Dodd PJ. (The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling). PLoS Med, 2016; 13(10): e1002152.
6. World Health Organization. (Global Tuberculosis Report 2025). Geneva; World Health Organization: 2025.
7. Thomas TA. (Tuberculosis in children). Pediatr Clin North Am, 2017; 64(4): 893-909.
8. Cantini F, Niccoli L, D'Angelo S, et al. (Risk of tuberculosis reactivation associated with biological therapeutics: TNF-alpha blockers versus non-TNF-alpha blockers). Autoimmun Rev, 2017; 16(11): 1184-1189.
9. McQuaid CF, McCreesh N, Read JM, et al. (The potential impact of COVID-19-related disruption on tuberculosis burden). Nat Med, 2021; 27(4): 654-660.
10. Steingart KR, Schiller I, Horne DJ, et al. (Xpert MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults). Cochrane Database Syst Rev, 2014; (1): CD009593.
11. Denkinger CM, Schumacher SG, Boehme CC, et al. (Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: a systematic review and meta-analysis). Eur Respir J, 2014; 44(2): 435-446.
12. Bahr NC, Nuwagira E, Evans EE, et al. (Diagnostic accuracy of Xpert MTB/RIF Ultra for tuberculous meningitis in HIV-infected adults: a prospective cohort study). Lancet Infect Dis, 2018; 18(1): 68-75.
13. Petruccioli E, Chiacchio T, Pepponi I, et al. (First characterization of the CD4 and CD8 T-cell responses to QuantiFERON-TB Gold Plus). J Infect, 2016; 73(6): 588-597.
14. Sotgiu G, Saderi L, Petruccioli E, et al. (QuantiFERON-TB Gold Plus for the diagnosis of tuberculosis: a systematic review and meta-analysis). J Infect, 2019; 79(6): 444-453.
15. Rozot V, Vigano S, Mazza-Stalder J, et al. (Mycobacterium tuberculosis-specific CD8+ T cells are functionally and phenotypically different between latent infection and active disease). Eur J Immunol, 2013; 43(6): 1568-1577.
16. Zegmout A, Boucaid A, Souhi H, et al. (La place du test Quantiféron dans la détection de la tuberculose latente avant traitement par les anti-TNF-alpha). Rev Mal Respir, 2017; 34(Suppl): A216.

17. Hu X, Zhang H, Li Y, et al. (Analysis of the diagnostic efficacy of the QuantiFERON-TB Gold In-Tube assay for preoperative differential diagnosis of spinal tuberculosis). Front Cell Infect Microbiol, 2022; 12: 983579.
18. Nikitina IY, Kondratuk NA, Kosmiadi GA, et al. (Mtb-Specific CD4 and CD8 T Cells Displays Different Phenotypic Profiles in Active vs. Latent Tuberculosis). J Immunol Res, 2016; 2016: 7265912.
19. Pourakbari B, Mamishi S, Benvari S, et al. (Comparison of the QuantiFERON-TB Gold Plus and QuantiFERON-TB Gold In-Tube interferon-γ release assays: A systematic review and meta-analysis). Adv Med Sci, 2019; 64(2): 437-443.
20. Qin T, Jie L, Ya W, et al. (The Diagnostic Efficacy of Interferon-Gamma Release Assays Combined with GeneXpert MTB/RIF Detection for Urinary Tract Tuberculosis). J Biosci Med, 2026; 14(01): 45-58.