

Comparison of PCR with Cytopathology in the Diagnosis of Tubercular Lymphadenitis

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Abstract

Background: Tubercular lymphadenitis is an important form of extrapulmonary tuberculosis and is a common cause of peripheral lymphadenopathy. Fine needle aspiration cytology (FNAC) is a simple and minimally invasive diagnostic method; however, cytological diagnosis and Ziehl-Neelsen staining may have limitations, particularly in paucibacillary and cytologically equivocal cases. Molecular techniques such as real-time polymerase chain reaction (PCR) may provide additional diagnostic information.

Objectives: To evaluate the diagnostic performance of FNAC in tubercular lymphadenitis in comparison with real-time PCR, and to determine the incidence, age and sex distribution of tubercular lymphadenitis among patients presenting with lymphadenopathy.

Methods: This cross-sectional study was conducted in the Department of Pathology, Gauhati Medical College and Hospital, Guwahati, from August 2014 to July 2015. A total of 217 patients with lymphadenopathy were evaluated by FNAC. Smears were examined following May-Grünwald Giemsa and Ziehl-Neelsen staining. Real-time PCR for detection of Mycobacterium tuberculosis DNA was performed on 102 specimens, comprising 87 cases diagnosed as tubercular lymphadenitis by FNAC and 15 cases showing necrotic material only. The diagnostic performance of FNAC was assessed using real-time PCR as the reference test.

Results: Tubercular lymphadenitis was diagnosed by FNAC in 87 of 217 cases (40.1%). The most commonly affected age group was 21–30 years, and there was a slight female predominance (51.7%). Cervical lymph nodes were the predominant site of involvement (94.3%). Granuloma with caseation necrosis was observed in 78.5% of cases. Ziehl-Neelsen staining demonstrated acid-fast bacilli in 58.6% of FNAC-diagnosed cases. Overall, real-time PCR was positive in 97 of 102 specimens (95.1%). PCR was positive in 86 of 87 FNAC-diagnosed cases (98.9%) and in 11 of 15 cases reported as necrosis only (73.3%). Using PCR as the reference test, FNAC demonstrated a sensitivity of 88.7%, specificity of 80.0%, positive predictive value of 98.9%, negative predictive value of 26.7% and overall diagnostic accuracy of 88.2%. The discordance between FNAC and PCR was predominantly due to FNAC-negative/PCR-positive cases and was statistically significant on McNemar's exact test ($P=0.006$).

Conclusion: FNAC remains a useful and effective method for the initial diagnosis of tubercular lymphadenitis. However, its diagnostic limitations are particularly evident in cytologically equivocal and paucibacillary cases. Real-time PCR provided additional diagnostic yield, including detection of Mycobacterium tuberculosis DNA in a substantial proportion of cases reported as necrosis only. Molecular testing may therefore serve as a valuable adjunct to FNAC in the diagnosis of tubercular lymphadenitis.

Keywords: Tubercular lymphadenitis; fine needle aspiration cytology; FNAC; polymerase chain reaction; real-time PCR; Ziehl-Neelsen staining; lymphadenopathy.

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Introduction

Tuberculosis (TB) remains a major global health problem and continues to cause substantial morbidity and mortality, particularly in high-burden countries. Although pulmonary disease is the most common manifestation, extrapulmonary tuberculosis represents an important proportion of the overall disease burden. Tuberculous

lymphadenitis is one of the commonest forms of extrapulmonary tuberculosis and frequently presents as peripheral, particularly cervical, lymphadenopathy. [1] Tuberculous lymphadenitis is characterized by chronic granulomatous inflammation of lymph nodes and may present with painless, discrete or matted lymph node

enlargement, with or without constitutional symptoms. The diagnosis may be challenging because clinical examination and radiological investigations alone may not establish the underlying aetiology of lymphadenopathy. Demonstration of acid-fast bacilli by smear microscopy provides specific evidence of mycobacterial infection; however, the sensitivity of direct microscopy may be limited, particularly in paucibacillary disease. The diagnosis may therefore require a combination of cytological, microbiological and molecular investigations. [1,2]

Fine needle aspiration cytology (FNAC) is a simple, rapid and minimally invasive technique for the evaluation of peripheral lymphadenopathy and has an established role in the diagnosis of tuberculous lymphadenitis. Characteristic cytomorphological findings, including epithelioid cell granulomas with or without caseous necrosis, may permit a diagnosis on cytological examination. However, the absence of characteristic granulomatous changes or the presence of necrotic material alone may make cytological diagnosis difficult. The sensitivity of Ziehl-Neelsen staining is also variable, particularly in paucibacillary specimens. These limitations create a role for additional diagnostic methods. [2,3]

Recent evidence continues to support the value of molecular testing in tuberculous lymphadenitis. In an Indian study comparing FNAC with GeneXpert, molecular testing demonstrated higher sensitivity than FNAC and acid-fast bacilli staining.³ More recent evidence has shown that combining FNAC, microscopy and molecular testing can further improve the rapid diagnosis of tuberculous lymphadenitis. [4]

The present study, entitled "Comparison of PCR with Cytopathology in the Diagnosis of Tubercular Lymphadenitis," was undertaken to evaluate the diagnostic performance of fine needle aspiration cytology in tubercular lymphadenitis in comparison with real-time PCR. The study also aimed to determine the incidence of tubercular lymphadenitis among cases of lymphadenopathy and to study the age and sex distribution of affected patients.

Materials and Methods

Study Design and Setting: This was a cross-sectional study conducted in the Department of Pathology, Gauhati Medical College and Hospital, Guwahati, Assam, over a period of one year from August 2014 to July 2015. The study was approved by the Institutional Ethical Committee of Gauhati Medical College and Hospital vide letter no. NO.MC/233/2013/334 dated 27 November 2014.

Patients of all age groups and both sexes attending the outpatient departments with lymphadenopathy

and clinical suspicion of tubercular pathology were considered for inclusion. A thorough clinical examination was performed before the procedure. Written informed consent was obtained from patients prior to FNAC, while consent was obtained from parents or guardians for minor patients.

Inclusion and Exclusion Criteria: Patients presenting with lymphadenopathy and clinical suspicion of tubercular pathology were included in the study. Patients in whom the diagnosis of tuberculosis was not confirmed, those who did not provide consent, and those in whom aspiration could not be performed were excluded.

Fine Needle Aspiration Cytology: Clinical assessment included evaluation of the number, size, shape, surface, consistency, mobility, fixity to the skin and underlying tissues, and tenderness of the lymph nodes. Relevant laboratory and radiological investigations, including haemoglobin, total leukocyte count, differential leukocyte count, erythrocyte sedimentation rate and chest radiography, were recorded. Ziehl-Neelsen staining was performed for detection of acid-fast bacilli.

FNAC was performed using a disposable 24–25 gauge needle attached to a 10-mL syringe; a 25-gauge needle was used in paediatric patients. The aspirated material was expressed onto clean, grease-free glass slides and smears were prepared. Depending on the nature of the aspirate, direct smearing or processing of fluid aspirates was performed. The smears were subjected to May-Grünwald Giemsa (MGG) staining and Ziehl-Neelsen staining. Methanol was used as the fixative for MGG-stained smears.

Cytological Examination: All smears were examined microscopically initially under low power to assess the overall cellular pattern and subsequently under high power for detailed cytomorphological evaluation. Ziehl-Neelsen-stained smears were examined under oil immersion for the presence of acid-fast bacilli. Cases diagnosed cytologically as tubercular lymphadenitis were categorized according to their cytomorphological pattern, including granuloma with caseation necrosis and granuloma without caseation necrosis.

DNA extraction and real-time PCR: FNAC material selected for molecular analysis was subjected to DNA extraction and purification using the QIAamp DNA extraction protocol (Qiagen). The extracted DNA was subsequently analyzed using the Care TBPCR kit for qualitative real-time PCR detection of *Mycobacterium tuberculosis* DNA. The assay uses nucleic acid amplification by polymerase chain reaction in combination with real-time fluorescent probe detection. The kit incorporates a dUTP-UNG system to minimize

carry-over contamination and has a stated detection limit of 10 MTB bacteria/mL. PCR reactions were prepared according to the manufacturer's protocol using TB PCR solution, Taq DNA polymerase and UNG enzyme, with appropriate negative and positive controls. Eighteen microlitres of the prepared master mix was aliquoted into each reaction tube, followed by addition of 2 μ L of extracted DNA or control material. Amplification and detection were performed using a 7500 Fast Dx Real-Time PCR instrument. The cycling protocol consisted of an initial incubation at 37°C for 5 minutes followed by 94°C for 1 minute, and 40 cycles of 95°C for 5 seconds and 60°C for 30 seconds, followed by a hold at 4°C. Samples with no CT value were reported as MTB negative, while samples with a CT value $\leq$ 37.0 were reported as MTB positive. Samples with CT values between 37.0 and 40.0 were retested according to the assay protocol; a sample was considered negative if the repeat test showed no CT value and positive otherwise.

Statistical Analysis: The diagnostic performance of FNAC was assessed by calculating sensitivity and specificity using real-time PCR results as the reference test.

Sensitivity was calculated as the proportion of true-positive results among all PCR-positive cases, while specificity was calculated as the proportion of true-negative results among all PCR-negative cases.

Results

A total of 217 patients with lymphadenopathy were enrolled during the study period. The age of the patients ranged from 1 year 5 months to 78 years. There were 128 male and 89 female patients. On FNAC, tubercular lymphadenitis was the most frequent diagnosis, accounting for 87 cases (40.1%), followed by reactive lymphadenitis (65; 30.0%), metastatic malignancy (30; 13.8%), necrosis only (15; 6.9%), suppurative lymphadenitis (10; 4.6%), poorly differentiated carcinoma (7; 3.2%) and lymphoma (3; 1.4%).

Table 1: Age distribution of cases according to FNAC diagnosis

Age group (years)	Tubercular lymphadenitis n (%)	Reactive lymphadenitis n (%)	Metastatic malignancy n (%)	Lymphoma n (%)	Suppurative lymphadenitis n (%)	Poorly differentiated carcinoma n (%)	Necrosis only n (%)	Total
0–10	7	20	1	2	2	0	1	33
11–20	17	16	0	0	3	0	4	41
21–30	35	7	1	0	2	0	6	50
31–40	15	12	4	0	1	0	3	35
41–50	9	9	9	0	0	2	0	29
51–60	3	1	5	0	1	4	0	14
61–70	1	0	7	1	1	0	1	11
71–80	0	0	3	0	0	1	0	4
Total	87	65	30	3	10	7	15	217

Among the 87 cases diagnosed as tubercular lymphadenitis by FNAC, the 21–30-year age group was predominant, comprising 35 cases (40.2%). The distribution of age groups differed significantly between patients with tubercular lymphadenitis and those with other FNAC diagnoses ($\chi^2 = 37.16$, df = 7, $P < 0.001$).

Demographic and Clinical Characteristics: Of the 87 FNAC-diagnosed cases of tubercular lymphadenitis, 45 (51.7%) were female and 42 (48.3%) were male. The difference from an equal

male-to-female distribution was not statistically significant (binomial test, $P \approx 0.83$), indicating no demonstrable sex predominance within the tubercular lymphadenitis group.

Cervical lymph nodes constituted the predominant site, accounting for 82 of 87 cases (94.3%), followed by axillary nodes (3; 3.4%) and inguinal nodes (2; 2.3%).

Among cervical tubercular lymphadenitis cases, discrete nodes were more frequent than matted nodes (57.3% vs 42.7%).

Table 2: Demographic and clinical characteristics of FNAC-diagnosed tubercular lymphadenitis

Characteristic	n	%
Sex		
Male	42	48.3
Female	45	51.7
Age group		
0–10 years	7	8.0
11–20 years	17	19.5
21–30 years	35	40.2
31–40 years	15	17.2
41–50 years	9	10.3
51–60 years	3	3.4
61–70 years	1	1.1
71–80 years	0	0
Site		
Cervical	82	94.3
Axillary	3	3.4
Inguinal	2	2.3
Posterior auricular	0	0
Cervical node morphology (n = 82)		
Discrete	47	57.3
Matted	35	42.7

Of the 87 FNAC-diagnosed cases, 68 (78.5%) showed granulomas with caseation necrosis, while 19 (21.5%) showed granulomas without caseation necrosis. Ziehl-Neelsen staining demonstrated acid-fast bacilli in 51 cases (58.6%), whereas 36 (41.4%) were negative.

Table 3: Cytomorphological pattern and Ziehl-Neelsen staining in tubercular lymphadenitis

Finding	n	%
Cytomorphological pattern		
Granuloma with caseation necrosis	68	78.5
Granuloma without caseation necrosis	19	21.5
Ziehl-Neelsen staining		
AFB positive	51	58.6
AFB negative	36	41.4

The proportion of AFB-positive cases was 58.6% (95% CI, approximately 47.6–69.1%), demonstrating that direct Ziehl-Neelsen staining failed to demonstrate AFB in a substantial proportion of cases diagnosed cytologically as tubercular lymphadenitis.

Real-time PCR findings: Real-time PCR was performed in 102 cases, comprising all 87 cases

diagnosed as tubercular lymphadenitis by FNAC and 15 cases in which only necrotic material was obtained. Overall, PCR was positive for *Mycobacterium tuberculosis* in 97 cases (95.1%).

Among the 87 FNAC-diagnosed cases, 86 (98.9%) were PCR positive and one (1.1%) was PCR negative. Among the 15 necrosis-only cases, 11 (73.3%) were PCR positive and four (26.7%) were PCR negative.

Table 4: Comparison of FNAC diagnosis with real-time PCR results

FNAC category	PCR positive n (%)	PCR negative n (%)	Total
Tubercular lymphadenitis	86 (98.9)	1 (1.1)	87
Necrosis only	11 (73.3)	4 (26.7)	15
Total	97 (95.1)	5 (4.9)	102

PCR positivity was significantly higher among cases diagnosed as tubercular lymphadenitis by FNAC than among those showing necrosis only (98.9% vs 73.3%; Fisher's exact test, $P = 0.001$).

Importantly, 11 of the 15 necrosis-only cases were PCR positive, indicating that molecular testing

identified *M. tuberculosis* DNA in a substantial proportion of cytologically equivocal specimens.

Diagnostic performance of FNAC: For the comparison of FNAC with PCR, the original study data yielded 86 true-positive, 4 true-negative, 1 false-positive and 11 false-negative results.

Table 5: showing Diagnostic performance of FNAC compared with real-time PCR

	PCR positive	PCR negative	Total
FNAC positive	86	1	87
FNAC negative	11	4	15
Total	97	5	102
Diagnostic parameter	Estimate		
Sensitivity	88.7%		
Specificity	80.0%		
Positive predictive value	98.9%		
Negative predictive value	26.7%		
Overall accuracy	88.2%		

The sensitivity of FNAC was 88.7% (95% CI, 80.6–94.2%) and specificity was 80.0% (95% CI, 28.4–99.5%). The positive predictive value was high at 98.9%, whereas the negative predictive value was comparatively low at 26.7%.

Because FNAC and PCR were performed on the same specimens, the discordant results were additionally assessed using McNemar's exact test. The 11 FNAC-negative/PCR-positive results compared with only one FNAC-positive/PCR-negative result demonstrated a significant asymmetry ($P = 0.006$). This indicates that the discrepancy between the two methods was predominantly attributable to cases in which PCR detected tuberculosis despite a negative FNAC result.

Discussion

Tuberculous lymphadenitis remains an important cause of peripheral lymphadenopathy, particularly in countries with a high burden of tuberculosis. In the present study, tubercular lymphadenitis was the most common diagnosis among the 217 cases of lymphadenopathy evaluated by fine needle aspiration cytology (FNAC), accounting for 87 cases (40.1%). This is consistent with earlier studies in which tuberculous lymphadenitis constituted a major proportion of patients presenting with cervical lymphadenopathy. Almobarak et al. reported tuberculous lymphadenitis as a frequent cause of lymphadenopathy in their prospective series, while Kulal et al. similarly identified tuberculosis as the commonest non-neoplastic cause of cervical lymphadenopathy.[5,6]

The 21–30-year age group was most frequently affected in the present study, accounting for 40.2% of tubercular lymphadenitis cases. The age distribution differed significantly between patients with tubercular lymphadenitis and those with other cytological diagnoses ($P < 0.001$), demonstrating a predominance of tuberculous disease among younger patients in this cohort. A similar predilection for young adults has been described in previous studies of tuberculous lymphadenitis.[5] There was a slight female preponderance in the present study, with females comprising 51.7% of

cases; however, this difference was not statistically significant. Almobarak et al. also reported a female predominance, with females constituting 61.2% of their study population.[5] Cervical lymph nodes were the predominant site of involvement, accounting for 94.3% of tubercular lymphadenitis cases. Among the cervical lymph nodes, Level V was the most frequently involved group. The predominance of cervical involvement is consistent with previous studies of tuberculous lymphadenitis.[5,6] Kulal et al. also reported the posterior triangle (Level V) as the most commonly involved cervical nodal group. [6] Among the cervical cases in the present study, 57.3% had discrete nodes and 42.7% had matted nodes. Kulal et al. similarly reported a predominance of discrete over matted lymph nodes.[6] These findings indicate that the classical clinical presentation of matted cervical lymph nodes is not invariably present in tuberculous lymphadenitis.

Granuloma with caseation necrosis was the predominant cytomorphological pattern, observed in 78.5% of cases, while granuloma without caseation necrosis was observed in 21.5%. Similar findings have been reported in earlier cytological studies. Almobarak et al. reported necrotizing granulomatous lymphadenitis in 88.3% of their cases.⁵ The predominance of granulomatous inflammation with necrosis in the present study supports the diagnostic value of this cytomorphological pattern in tuberculous lymphadenitis.

Acid-fast bacilli were demonstrated by Ziehl-Neelsen staining in 58.6% of FNAC-diagnosed cases. Thus, despite characteristic cytomorphological features, approximately 41% of cases did not demonstrate AFB on direct microscopy. Almobarak et al. reported AFB positivity in 47% of their cases, while Dhawan et al. observed that AFB positivity was greatest in lesions showing granulomatous inflammation with necrosis.[5,7] These findings highlight the limited sensitivity of direct AFB microscopy and the importance of correlating Ziehl-Neelsen staining with cytomorphological findings, particularly in paucibacillary disease. Real-time PCR provided substantial additional diagnostic information in the

present study. Of the 102 specimens subjected to molecular testing, 97 (95.1%) were positive for *Mycobacterium tuberculosis* DNA. Among the 87 cases diagnosed as tubercular lymphadenitis by FNAC, 86 (98.9%) were PCR positive. More importantly, 11 of the 15 cases (73.3%) reported as necrosis only on cytology were also PCR positive. PCR positivity was significantly different between the FNAC-diagnosed tubercular lymphadenitis and necrosis-only groups ($P=0.001$). The high PCR positivity among necrosis-only aspirates is clinically relevant. Necrotic aspirates lacking definite epithelioid granulomas may be difficult to classify as tuberculous on cytomorphology alone, potentially resulting in false-negative cytological diagnoses. Gupta V et al., in a comparative study of real-time PCR and culture in tubercular lymphadenopathy, similarly demonstrated the usefulness of real-time PCR in detecting *M. tuberculosis* in lymph-node specimens. [8] The present finding therefore supports molecular testing in patients with clinically suspected tuberculous lymphadenitis when FNAC yields predominantly necrotic material without characteristic granulomatous inflammation.

More recent molecular studies have reinforced this observation. Gouda et al., in an Indian study comparing FNAC with GeneXpert for tuberculous lymphadenitis, reported sensitivities of 72.17% for FNAC and 83.04% for CBNAAT against a composite reference standard, demonstrating the additional diagnostic yield provided by molecular testing. [3] Although GeneXpert and the real-time PCR assay employed in the present study represent different molecular platforms, both studies demonstrate the potential value of nucleic acid amplification in cytologically inconclusive cases.

Using real-time PCR as the reference test, FNAC demonstrated a sensitivity of 88.7%, specificity of 80.0% and overall diagnostic accuracy of 88.2% in the present study. The positive predictive value was 98.9%, indicating that cases diagnosed cytologically as tubercular lymphadenitis were highly likely to be PCR positive within the study population. In contrast, the negative predictive value was only 26.7%, indicating that the absence of diagnostic cytomorphological features could not reliably exclude tuberculous lymphadenitis.

This observation was further supported by analysis of the discordant FNAC and PCR results. Eleven cases were FNAC negative but PCR positive, whereas only one case was FNAC positive and PCR negative.

The asymmetry of these discordant results was statistically significant on McNemar's exact test ($P=0.006$). Thus, disagreement between the two methods predominantly occurred because molecular testing detected *M. tuberculosis* in cases

not identified as tuberculous by cytology. These findings support the complementary rather than competing roles of cytomorphology and molecular testing. The diagnostic approach to tuberculous lymphadenitis has evolved considerably since the present study was conducted. Xpert MTB/RIF and the subsequently developed Xpert MTB/RIF Ultra provide rapid molecular detection of *M. tuberculosis*, together with information on rifampicin resistance. Recent studies have demonstrated improved diagnostic sensitivity with these molecular platforms in lymph-node tuberculosis. Yu et al., in a prospective head-to-head study, reported that Xpert MTB/RIF Ultra outperformed conventional Xpert in the diagnosis of tuberculous lymphadenitis, with a sensitivity of 81.0% for Ultra compared with 71.4% for Xpert on fine-needle aspirate specimens. [9] Minnies S et al. similarly demonstrated high sensitivity of Xpert Ultra in fine-needle aspiration biopsy specimens from patients with tuberculous lymphadenitis. [1]

These advances are reflected in current international recommendations. The World Health Organization now recommends low-complexity automated nucleic acid amplification tests on lymph-node aspirates as initial diagnostic tests for suspected extrapulmonary tuberculosis. [2] Current diagnostic practice therefore increasingly integrates molecular testing with conventional cytological, histopathological and microbiological assessment rather than relying on any single diagnostic modality.

An important consideration in interpreting the diagnostic performance observed in the present study is that real-time PCR was used as the reference test for calculating the sensitivity and specificity of FNAC. Molecular positivity itself should not be regarded as an absolute gold standard for lymph-node tuberculosis. Diagnostic accuracy estimates vary according to whether microbiological or composite reference standards are employed, and this remains an important consideration when comparing results across different studies. [2] Consequently, the high positive predictive value and relatively low negative predictive value observed in the present study should be interpreted within the context of the selected study population and reference standard.

The present study demonstrates that FNAC remains a useful, rapid and minimally invasive method for the initial evaluation of tuberculous lymphadenitis. Characteristic granulomatous inflammation with caseation necrosis provided a cytological diagnosis in the majority of cases; however, direct demonstration of AFB was considerably less frequent. Molecular testing provided additional diagnostic yield, particularly in aspirates showing

necrosis without definitive granulomatous inflammation.

The significant excess of FNAC-negative/PCR-positive cases further demonstrates the potential for molecular testing to identify disease that may be missed by cytomorphology alone. Integration of cytological assessment with molecular testing may therefore provide a more effective diagnostic approach to tuberculous lymphadenitis, particularly in cytologically equivocal and paucibacillary cases.

Conclusion

Tubercular lymphadenitis was the most common diagnosis among the 217 cases of lymphadenopathy evaluated in the present study. It predominantly affected young adults, with a slight female preponderance, and the cervical lymph nodes were the most frequently involved site. FNAC demonstrated characteristic cytomorphological features in the majority of cases and showed good diagnostic sensitivity and accuracy. However, Ziehl-Neelsen staining was positive in only 58.6% of FNAC-diagnosed cases, highlighting the limitation of direct AFB detection.

Real-time PCR demonstrated additional diagnostic value, particularly in cytologically equivocal cases. Of the 15 cases reported as necrosis only on FNAC, 11 (73.3%) were PCR positive. FNAC showed a sensitivity of 88.7%, specificity of 80.0% and overall accuracy of 88.2% when compared with real-time PCR as the reference test. The significantly greater number of FNAC-negative/PCR-positive cases further supports the complementary role of molecular testing.

The findings suggest that real-time PCR can serve as a useful adjunct to FNAC in the diagnosis of tubercular lymphadenitis, particularly in paucibacillary and cytologically inconclusive cases. Combining cytomorphological assessment with molecular testing may improve diagnostic yield and facilitate earlier confirmation of tuberculous lymphadenitis.

Limitations: The study was conducted at a single tertiary-care centre and was hospital based, limiting the generalizability of the findings to the wider population. The number of specimens subjected to PCR was relatively small, particularly the PCR-negative group, which resulted in wide confidence intervals for specificity and negative predictive value.

Real-time PCR was used as the reference test for the comparative analysis; culture and histopathological confirmation were not available for all cases.

Therefore, the diagnostic performance estimates should be interpreted within the context of the reference method used in this study. The molecular

assay evaluated was also an earlier real-time PCR platform, and its performance should not be directly equated with that of currently available molecular tests such as Xpert MTB/RIF Ultra.

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