ORIGINAL ARTICLE

Evaluation of the Xpert MTB/RIF Performance on Tissues: Potential Impact on Airborne Infection Isolation at a Tertiary Cancer Care Center

Tracy McMillen, BS;[1] Shauna C. Usiak, MPH;[2] Liang Hua Chen, BS;[1] Luz Gomez, MPH;[1] Peter Ntiamoah, MPH;[3] Meera R. Hameed, MD;[3] Indre Budvytiene, BS;[4] Niaz Banaei, MD;[4] Mini Kamboj, MD;[2,5] N. Esther Babady, PhD[1,5]

OBJECTIVES. In this study, we sought to evaluate the performance of the Xpert MTB/RIF (Cepheid) assay for the detection of Mycobacterium tuberculosis (MTB) complex DNA on fresh and formalin-fixed, paraffin-embedded (FFPE) tissue specimens from oncology patients in an area with a low prevalence of tuberculosis. We also aimed to retrospectively assess the potential impact of Xpert MTB/RIF on the duration of airborne infection isolation (AII).

SETTING. A 473-bed, tertiary-care cancer center in New York City.

DESIGN. A total of 203 tissue samples (101 FFPE and 102 fresh) were tested using Xpert MTB/RIF, including 133 pulmonary tissue samples (65.5%) and 70 extrapulmonary tissue samples (34.5%). Acid-fast bacilli (AFB) culture was used as the diagnostic gold standard. The limit of detection (LOD) and reproducibility were also evaluated for both samples types using contrived specimens. The potential impact of the Xpert MTB PCR assay on tissue samples from AII patients on AII duration was retrospectively assessed.

RESULTS. Using the Xpert MTB/RIF for fresh tissue specimens, the sensitivity was 50% (95% CI, 1.3%–98.7%) and the specificity was 99% (95% CI, 94.5%–99.9%). For FFPE tissue specimens, the sensitivity was 100% (95% CI, 63.1%–100%) and the specificity was 98.3% (95% CI, 95.5%–100%. The LOD was 10^3 colony-forming units (CFU)/mL for both fresh and FFPE tissue specimens, and the Xpert MTB/RIF was 100% reproducible at concentrations 10 times that of the LOD. With an expected turnaround time of 24 hours, the Xpert MTB PCR could decrease the duration of AII from a median of 8 days to a median of 1 day.

CONCLUSIONS. The Xpert MTB/RIF assay offers a valid option for ruling out *Mycobacterium tuberculosis* complex (MTBC) on tissue samples from oncology patients and for minimizing AII resource utilization.

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The Xpert MTB/RIF (Cepheid, Sunnyvale, CA) has been cleared by the US Food and Drug Administration (FDA) for the detection of *M. tuberculosis* (MTB) complex DNA directly on raw sputum or concentrated sediments prepared from induced or expectorated sputum.[1] The Xpert MTB/RIF assay can also detect resistance to rifampin (RIF) in samples positive for MTB complex DNA. In 2015, the Xpert MTB/RIF received additional FDA clearance for use as an alternative to acid-fast bacilli (AFB) smears on sputum samples for removing patients from airborne infection isolation (AII).[1]

Patients with a presumptive or confirmed diagnosis of cancer often undergo biopsy sampling as part of their initial diagnostic evaluation or during the process of disease restaging. Respiratory tissue samples account for the vast majority of AFB-positive samples or those depicting granulomatous changes. Among US-born patients, incidental identification of AFB in tissue biopsies, without concomitant risk factors for TB, is a common reason for infectious diseases consultation, prompting additional testing, particularly requests to rule out MTB. More frequently, original fresh biopsy samples are unavailable, excluding AFB culture as an option. In these situations, FFPE or, rarely, residual fresh tissue samples may be the only specimens available with which microbiologists can undertake additional testing. In healthcare settings with low

Affiliations: 1. Clinical Microbiology Service, Department of Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, New York; 2. Infection Control Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York; 3. Surgical Pathology Service, Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, New York; 4. Department of Pathology and Laboratory Medicine, Stanford University Medical Center, Palo Alto, California; 5. Infectious Disease Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York.

PREVIOUS PRESENTATION. The results of this study were presented in part at the 2016 American Society of Microbiology Microbe Meeting on June 20, 2016, in Boston, Massachusetts.

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tuberculosis (TB) prevalence, a prompt resolution on the need for AIIR for these cases is vital.

Currently, data on the performance of the Xpert MTB/RIF on fresh and FFPE tissue samples are limited. If the high negative predictive value (NPV) of the Xpert MTB/RIF assay is established for these samples, a considerable reduction in resources utilized for instituting and maintaining AII precautions could be achieved. In addition, patient need for AII, empiric TB therapy, or repeat invasive diagnostic procedures could be avoided. These aims comprise the basis of our current study.

We evaluated the performance of the Xpert MTB/RIF on FFPE and fresh tissue samples at a tertiary-care cancer center with low rates of TB and rising prevalence of nontuberculous mycobacteria (NTM), and we assessed the potential impact of rapid Xpert MTB/RIF results from tissue samples on the duration of AII.

MATERIALS AND METHODS

Study Setting

Memorial Sloan Kettering Cancer Center (MSKCC) is a 473-bed, tertiary-care cancer where >20,000 biopsies are performed each year. Mycobacterial special stains (eg, Fite stains) are performed based on the presence of inflammation or granulomas. Each year, these special stains are performed for ~680 cases.

In the 5 years from 2011 to 2015, an average of 156 patients per year were placed in AII for suspected TB (range, 96–218 patients). Moreover, 29 cases of TB were diagnosed during this period. NTM account for the vast majority of AFB, dominated by Mycobacterium avium complex (MAC) (average 80 cases per year). Airborne infection isolation (AII) at our institution is implemented based on clinical suspicion for TB and/or positive AFB smear or culture from a respiratory sample, including lung tissue biopsies.

Samples

Clinical samples included in the study were fresh and FFPE tissue samples submitted to the Clinical Microbiology Laboratory or the Surgical Pathology Laboratory at MSKCC from 2010 to 2016. An additional 4 FFPE samples, positive for MTB by real-time PCR and culture on the corresponding fresh tissue samples (Stanford University, Palo Alto, CA)[2] were obtained to further test the sensitivity of the Xpert MTB/RIF, for a total of 101 FFPE samples. Fresh tissue specimens were stored at −80°C following initial AFB culture set-up. The MSKCC Institutional Review Board granted a waiver of informed consent and HIPAA authorization for this study (IRB no. 15–119).

Tissue Processing

Fresh tissue specimens (~0.5 cm^2) and FFPE tissue specimens (5–10-µm sections) were processed as previously described.[3] The following day, 500 µL digested tissue was mixed with 1.5 mL sample treatment buffer, mixed vigorously in the vortex machine, incubated for 15 minutes at room temperature, and then transferred to the Xpert MTB/RIF cartridge. The Xpert MTB/RIF polymerase chain reaction (PCR) assay was conducted according to the manufacturer's instructions.

Limit of Detection (LOD)

The *Mycobacteria* growth indicator tube (MGIT) samples positive for MTB were used for spiking studies. An MTB-positive MGIT sample with a cycle threshold (Ct) value of 20 for probe A (~10^8 CFU/mL, based on colony count) was made with PCR-grade water and used as the stock solution for all series of 10-fold dilutions in the appropriate matrices. Pools of previously tested (ie, negative) fresh and FFPE tissue specimens were used for spiking studies. For FFPE tissue samples, after the xylene/ethanol wash, 100 µL proteinase K (20 mg/mL), 50 µL 10% SDS, and 350 µL 1X TBE were added to each tube. Starting with ~ 10^6 CFU/mL dilution stock, a series of 10-fold dilutions were made (in deparaffinized tissue pool) up to a concentration of ~ 10^2 CFU/mL, and all samples were tested in duplicate. Samples were mixed overnight at 500 rpm/55°C. For fresh tissue samples, 500 µL tissue pool was added to a microcentrifuge tube with 100 µL proteinase K (20 mg/mL), 50 µL 10% SDS, and 430 µL 1X TBE. A series of 10-fold dilutions were made similarly for fresh tissue samples. Samples were mixed overnight at 500 rpm at 55°C and were tested the next day in duplicate. For comparison, similar experiments were performed in parallel using spiked sputa, the FDA-cleared sample type.

Reproducibility

Reproducibility was determined by analyzing replicate testing of the same spiked tissue specimens at 2 concentrations on the same day: ~ 10^3 CFU/mL and ~ 10^4 CFU/mL. The average and standard deviation (SD) of the Ct values were recorded to establish reproducibility. In addition to the agreement between repeat, the average, SD, and coefficient of variation (% CV) of the Ct values were calculated as a measure of reproducibility across both tissue matrices. For comparison, similar experiments were performed in parallel using spiked sputa, the FDA-cleared sample type.

Impact on Airborne Infection Isolation

Clinical information was extracted from the medical records of patients for whom an MTB polymerase chain reaction (PCR) test was ordered to rule out TB. Because testing of the Xpert MTB/RIF was performed retrospectively, a hypothetical impact was estimated in comparison to results obtained with a reference-laboratory, laboratory-developed test (LDT) MTB PCR (Mayo Medical Laboratories, Rochester, MN) and/or culture results. Data collected included demographic information, clinical diagnosis, laboratory data, hospital location, and duration of AII.

RESULTS

Test Performance

Overall, 102 fresh tissue samples were included in the evaluation: 68 pulmonary tissue samples (66.7%) and 34 extrapulmonary

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TABLE 1. Accuracy of Xpert MTB/RIF on Fresh Tissues[a]

	M. tuberculosis Complex Culture		
Xpert MTB/RIF	Positive	Negative	Total
Positive	1	1[b]	2
Negative	1	99	100
Total	2[c]	100[d]	102

NOTE. MTB/RIF, *Mycobacterium tuberculosis* DNA and resistance to rifampicin; LDT PCR, laboratory-developed test polymerase chain reaction assay; AFB, acid-fast bacilli; CI, confidence interval.
[a]Sensitivity: 50% (1/2 samples; 95% CI, 1.2%–98.7%); specificity: 99% (99/100 samples; 95% CI, 94.5%–99.6%); positive predictive value: 100% (1/2 samples; 95% CI, 16%–100%); negative predictive value: 98% (99/100 samples; 95% CI, 90%–99.9%).
[b]Confirmed positive by a LDT PCR.
[c]2/2 samples were AFB smear negative.
[d]2/99 samples were AFB smear positive.

TABLE 2. Accuracy of Xpert MTB/RIF on FFPE Tissues[a]

	M. tuberculosis Complex Culture		
Xpert MTB/RIF	Positive	Negative	Total
Positive	7	1[b]	8[c]
Negative	0	52[d]	52[e]
Total	7[c]	53	60

NOTE. MTB/RIF, *Mycobacterium tuberculosis* DNA and resistance to rifampicin; LDT PCR, laboratory-developed test polymerase chain reaction assay; AFB, acid-fast bacilli; CI, confidence interval.
[a]Sensitivity: 100% (7/7 samples; 95% CI, 59%–100%), specificity: 98.1% (52/53 samples; 95% CI, 95.5%–100%), positive predictive value: 87.5% (7/8 samples; 95% CI, 63.1%–100%), negative predictive value: 100% (52/52 samples; 95% CI, 96.1%–99.9%).
[b]Confirmed positive by LDT PCR.
[c]6/8 Fite stain positive and 2/8 Fite stain negative samples.
[d]Additional samples were tested but not included in performance calculations: 7 samples with no AFB culture but negative by LDT PCR and 34 Xpert PCR negative samples with no AFB culture/LDT PCR. Results considered true negative results based on clinical history. A total of 93 samples were Xpert PCR negative.
[e]48/93 Fite stain negative, 43/93 Fite stain positive, 2/93 no Fite stain samples.

tissue samples (eg, arm, stomach, bones) (33.3%). Furthermore, 2 samples were AFB smear positive, and 14 were AFB culture positive: 10 MAC, 2 MTB, 1 *M. xenopi*, and 1 *M. fortuitum*. The Xpert MTB/RIF PCR was positive for 2 fresh tissue samples: 1 was positive by the LDT MTB PCR (but negative by culture) and 1 was positive for MTB on the corresponding culture. One tissue sample was negative by Xpert MTB/RIF but positive for MTB by culture. All other tissue specimens tested were negative for both Xpert MTB/RIF and culture. Sensitivity was 50% (95% CI, 1.2%–98.7%), specificity was 99% (95% CI, 94.5%–99.9%), positive predictive value (PPV) was 50% (95% CI, 1.2%–98.7%), and negative predictive value (NPV) was 99% (Table 1).

We tested 101 FFPE tissue blocks with the Xpert MTB/RIF PCR, including 65 pulmonary (64%) and 36 (36%) extrapulmonary FFPE tissue samples (eg, arm, stomach, bones). The Fite stain results were available for 99 samples (49 positive and 50 negative samples). Of 101 samples, 67 had a corresponding AFB culture (n = 60) or only the LDT MTB PCR (n = 7). Of 60 AFB cultures, 25 were positive for NTM (19 MAC, 2 *M. xenopi*, 1 *M. haemophilum*, 1 *M. kansasii*, 1 *M. abscessus*, and 1 *M. heckeshornense*). Of 101 samples, 34 had no AFB culture or LDT PCR performed. The Xpert MTB/RIF was positive for 8 FFPE tissue samples; all were confirmed by AFB culture and/or LDT TB PCR. All FFPE tissue samples with corresponding cultures and/or LDT MTB PCR negative for MTB were also negative by Xpert MTB/RIF. Of 34 samples, 31 were Fite stain negative and 3 were Fite stain positive. Medical charts were reviewed for all 34 samples (32 patients) to assess the presence of TB. None of these patients developed symptoms of TB. Results of all 34 samples were considered true-negative results. Overall, sensitivity was 100% (95% CI, 59%–100%), specificity was 98.1% (95% CI, 91%–99.9%), PPV was 87.5% (95% CI, 47.3%–99.7%), and NPV was 100% (93.4%–100%) (Table 2).

The LODs of the Xpert MTB/RIF in fresh and FFPE tissue samples were similar to that of induced sputum, as determined by parallel spiking studies in contrived samples (Tables S1 and S2). In all specimen types, the LOD was ~ 10^3 CFU/mL. There were no differences in % CV between the interassay and intraassay reproducibility data (data not shown). All % CV were within the ranges established by the manufacturers for sputum specimens (Tables S3 and S4).

Impact on Airborne Infection Isolation

At our institution, AII is implemented based on clinical suspicion and/or a FFPE tissue positive by Fite stain on respiratory tissue. During our study period, 21 Fite-positive FFPE respiratory samples underwent additional testing with LDT MTB PCR and/or culture. Xpert MTB/RIF PCR results were 100% concordant with the LDT TB PCR. An AFB culture was available on simultaneously collected fresh tissue specimens for 16 of 21 patients; 10 were positive for NTM, and 9 among these were eventually identified as MAC. Additional AFB cultures were set up from an alternate respiratory sample within 30 days of biopsy for 13 of 21 patients (sputum or bronchial washings or bronchoalveolar lavage). All were negative for MTB and 3 were positive for MAC.

In this study, the median patient age was 69 (range, 45–86 years old), and 14 (58%) were women. Pulmonary radiographic findings with nodular or cavitary changes were seen among 20/21 patients. In addition, 13 patients were hospitalized at the time of testing and 20 patients were placed on AII for a cumulative duration of 150 days (Table 3).

The median turnaround time to results for testing performed in real-time at the time of the study was 5 days (median, 5 days; range, 1–10 days). With the expected

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TABLE 3. Real-Time Testing to Remove Patient From Airborne Infection Isolation (AII)

Patient	Gender	Age	TB Exposure	Symptoms	Oncology	Source	Fite	TB PCR	Tissue		BAL/BW		Days in AII
									Smear	Culture	Smear	Culture	
1	M	58	No	None	None; cavitary lesion of CT	P	+	−	−	MAC	−	MAC	6
2	F	68	No	None	Breast; lung nodule	P	+	−	−	MAC	−	MAC	5
3	F	64	No	None	CML; lung nodule	P	+	−	−	MAC	−	MAC	8
4	F	60	No	None	None; lung nodule	P	+	−	−	−	−	−	8
5	F	70	No	Cold	Lung cancer	P	+	−	−	−	−	−	7
6	F	73	No	None	Breast	P	+	−	−	MAC	ND	ND	6
7	F	69	No	None	None; lung nodule	P	+	−	−	MAC	ND	ND	8
8	F	82	No	None	Smoker; Lung nodule	P	+	−	−	MAC	−	MAC	6
9	F	45	No	None	Colorectal; metastases to lung	P	+	−	ND	ND	ND	ND	6
10	M	55	No	None	None; lung nodule	P	+	−	−	MAC	ND	ND	8
11	F	63	No	None	Liver; lung nodule	P	+	−	ND	ND	ND	ND	1
12	M	86	No	None	Bladder	EP	ND	−	−	−	ND	ND	5
13	F	61	No	Sweat/dry cough	None; past MAC	P	+	−	−	−	ND	ND	9
14	M	84	No	None	None	P	+	−	−	MAC	−	MAC	8
15	M	70	No	None	Ear cell carcinoma; opacity	P	+	−	−	MAC	ND	ND	6
16	F	84	No	None	Lymphoma; lung nodule	P	+	−	−	−	ND	ND	7
17	M	53	Yes	Fever/ cough	Myeloproliferative neoplasm; multiple nodules	P	−	+	−	**MTB**	−	−	8
18	M	56	No	None	Lung nodule	P	+	+	−	−	ND	ND	4
19	F	61	No	None	Lung carcinoma	P	+	−	ND	ND	ND	ND	9
20	F	75	No	None	Lung cancer; lung nodule	P	+	−	+	MAC	ND	ND	14
21	F	73	No	None	None; lung nodule	P	−	−	ND	ND	ND	ND	8

NOTE. TB, tuberculosis; PCR, polymerase chain reaction; F, female; M, male; P, pulmonary; MAC, *Mycobacterium avium* complex; EP, extrapulmonary; +, positive; −, negative; ND, not done; OP, outpatient; IP, Inpatient; BAL, bronchoalveolar lavage fluid; BW, bronchial washings; AII, airborne infection isolation.

turnaround time for the Xpert MTB/RIF PCR of 1 day (2 days if received after hours when tissue processing is not performed) and the assumption that results would immediately be used to discontinue AII, we estimated a decrease in time in AII from a median of 8 days (range, 1–14 days; mean, 7.1 days; 95% CI, 6.0–8.3 days) to a median of 1 day (range, 1–2 days; mean, 1.1 days; 95% CI, 0.98–1.3 days).

DISCUSSION

This is the largest study to date evaluating the performance characteristics of the Xpert MTB/RIF on FPPE and fresh tissue samples from oncology patients. The most important findings of our study are the high specificity and high NPV of the assay in our low TB-prevalence setting. With its rapid turnaround time, results of the Xpert MTB/RIF can be used for early discontinuation of AII, particularly for patients with low risk factors and smear- or Fite-positive tissue samples.

Studies evaluating the performance of the Xpert MTB/RIF on tissue samples in high and low TB-burden areas have been published with sensitivity ranging from 30% to 88% and specificity ranging from 78% to 100%.[4–8] A study by Polepole et al[6] reported the performance of the Xpert MTB/RIF on 100 FFPE tissue samples, exclusively extrapulmonary samples, collected from patients at the University Teaching Hospital in Lusaka, Zambia.[6] The authors showed sensitivities ranging from 30% to 35% and specificities ranging from 78% to 94%. The difference in performance compared to our results could be attributed to sample storage, age of the FFPE blocks, and/or processing time prior to testing by the Xpert MTB/RIF test. Notably, that study used histopathology as the gold standard, which is not specific for MTBC. In our study, 67% of FFPE tissue samples had a corresponding fresh tissue sample with available AFB culture results. Except for one positive sample, results of the Xpert MTB/RIF PCR were concordant with AFB culture results. In a study from Mozambique, Xpert MTB/RIF performed on postmortem FFPE pulmonary tissue samples showed a sensitivity of 87.5% (95% CI, 47.3%–99.7%) and a specificity of 95.7% (95% CI, 78.1%–99.9%), a performance closer to that observed in our study for FFPE tissue samples.[4] A study performed in Spain, a low TB-prevalence area, and exclusively done on smear-negative samples compared the Xpert MTB/RIF to AFB culture on 12 tissue samples and reported a sensitivity of 41.7% and a specificity of 100%.[9] Our study included 100 smear-negative fresh tissue samples with a sensitivity of 50%, highlighting the importance of AFB culture, particularly for patients with appropriate risk factors. Our study is unique and different from these previous reports in

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that it includes many samples (>150) in a high-income, low TB-prevalence setting and an oncology patient population with low pretest probability for TB. In our large cohort of patients, the negative predictive value of the Xpert MTB PCR/RIF on smear-positive fresh and FFPE tissue samples was 100%.

Studies investigating the utility of the Xpert MTB/RIF PCR results from tissue specimens on the length of AII have not been performed. In oncology patients, incidental findings of lung lesions from routine or surveillance imaging studies often result in biopsy to rule out primary cancer or possible metastases. In this context, the pretest probability for TB is low, and the availability of a rapid test to rule-out TB from either fresh or paraffin-embedded tissue specimens would be ideal. A rapid result from the Xpert MTB/RIF assay could positively impact patient flow within the hospitals, including the time patients spend in AII as well as the patients' ability to make or keep outpatient appointments. In this study, we showed that the use of the Xpert MTB/RIF PCR on tissue samples could potentially decrease the time patients are kept in AII by a median of 7 days.

Our study has a few limitations. First, the overall number of positive samples was small compared to studies performed in areas with a high TB burden. However, our findings emphasize the utility of the assay to rule out TB in our low TB-prevalence setting and, as such, the high NPV of the test. Notably, the sensitivity and specificity established in this study were in line with those of other studies performed using samples from high TB-burden areas. Second, the LOD and reproducibility of the assay were determined using contrived samples, which are challenging to create for tissue samples, and our data may overestimate the modified assay performance characteristics. Third, cultures were not performed for all FFPE tissue samples; hence, it is possible that some of these patients may have had undiagnosed TB. Finally, the impact on AII was a hypothetical impact based on an expected turnaround time common to other tests performed in our laboratory on the GeneXpert. Further prospective studies are ongoing and will help determine the true impact of the Xpert MTB/RIF performance for tissue specimens from AII patients on AII duration.

In conclusion, our study confirms that the Xpert MTB/RIF can be performed on both FFPE and fresh tissue specimens and can provide data on the limit of detection and the reproducibility of the assay for tissue specimens. More importantly, we have established the potential role of the Xpert MTB/RIF in adequately ruling out *M. tuberculosis* in a low TB-prevalence cancer hospital, allowing patients to be safely removed from airborne infection isolation.

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Address correspondence to N. Esther Babady, PhD, D (ABMM), Clinical Microbiology Service, Department of Laboratory Medicine, Memorial Sloan Kettering Cancer Center, 327 East 64th Street, CLM 522, New York, New York (BabadyN@mskcc.org).

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit https://doi.org/10.1017/ice.2018.7

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