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Evaluating diagnostic performance of Truenat MTB Plus for gastrointestinal tuberculosis

Kusum Sharma,* Megha Sharma,*,† Vishal Sharma,‡ Megha Sharma,‡ Jayanta Samanta,‡ Aman Sharma,§ Rakesh Kochhar‡ and Saroj Kant Sinha‡

Departments of *Medical Microbiology, ‡Gastroenterology, §Internal Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, and †Department of Microbiology, All India Institute of Medical Sciences, Bilaspur, India

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Correspondence
Dr Kusum Sharma, Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India.
Email: sharmakusum9@yahoo.co.in

Kusum Sharma, Megha Sharma (MS1), and Vishal Sharma contributed equally as first authors.
Megha Sharma (MS1) was affiliated to 1 at the start and to 2 by the end of the study.

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Abstract

Background and Aim: Prompt and accurate diagnosis of gastrointestinal tuberculosis (GITB) along with simultaneous detection of drug resistance is inevitable for tuberculosis elimination. Truenat MTB Plus (TruPlus), a chip-based real-time polymerase chain reaction assay, was evaluated for the first time for diagnosing GITB and detecting rifampicin resistance.

Methods: Fifty ileocecal biopsy specimens (5 microbiologically confirmed GITB [culture-positive], 25 clinically confirmed GITB [culture-negative], and 20 control patients) processed in the Department of Microbiology between 2011 and 2021 were subjected to TruPlus assay, Xpert MTB RIF assay multiplex polymerase chain reaction. Their performance was evaluated against both culture and composite reference standard.

Results: The overall sensitivity and specificity of TruPlus in diagnosing GITB was 70% (21/30) and 100%, respectively. The sensitivity was 60% (3/5) for microbiologically confirmed cases and 72% (18/25) for clinically confirmed cases. Performance of TruPlus was superior to Xpert (sensitivity = 30%; P = 0.001) and comparable with MPCR (sensitivity = 83.33%; P = 0.13). Both TruPlus and MPCR had moderate agreement with reference standards, and MPCR detected additional three cases. Both TruPlus and Xpert correctly reported Rifampicin resistance in three cases.

Conclusions: TruPlus, with its greater portability and higher sensitivity than Xpert, could serve as an important tool for diagnosing GITB and rifampicin resistance at outreach endemic areas.

Introduction

Tuberculosis (TB) with its varied clinical presentations has been haunting mankind since times immemorial. TB was the leading cause of death due to infectious disease in the world in 2019,[1] and India contributed the largest share of TB cases (27%).[2] Further, there was a 10% increase in multidrug-resistant TB from 2018 to 2019.[3] Timely detection of TB along with presence of drug resistance could serve as keystone steps towards global TB elimination.

Gastrointestinal tuberculosis (GITB) contributes 10% of all extrapulmonary TB (EPTB) cases.[4] The mortality in GITB varies between 8% and 50%, and it has considerable morbidity in the form of intestinal ulcerations, altered bowel habits, and intestinal perforation.[4,5] In fact, GITB is an important cause of small intestinal perforation in India, second only to typhoid.[6] The most important challenge faced in a patient of GITB is the overlapping of symptoms with various other infectious and noninfectious diseases. The biochemical and radiological investigations being suboptimal,[7–9] it is the microbiological evidence of the tubercle bacilli that clinches the diagnosis of GITB.

Nucleic acid amplification tests (NAATs), with their rapidity and accuracy, have revolutionized the diagnosis of GITB over and above the conventional methods of smear and culture.

Polymerase chain reaction (PCR) including multiplexed PCR (MPCR),[10–12] isothermal assays,[13] and so forth detect *Mycobacterium tuberculosis* with high sensitivity, although presence of drug resistance is not detected. GeneXpert (Xpert), a semi-automated commercial platform, allows simultaneous detection of *M. tuberculosis* and rifampicin resistance. Although Xpert, under the aegis of National Tuberculosis Elimination Program, has been introduced widely into the Indian health-care system, its inherent requirement for a controlled temperature makes it unsuitable for outreach health-care centers.

Truenat MTB Plus (TruPlus), the second generation of Truenat MTB, has been developed indigenously in India as a small, chip-based NAAT for diagnosis of TB and detection of drug resistance.[14] It is portable and run on batteries. Its convenient size allows its usage at outreach centers with difficult terrain and road/electricity connectivity. TruPlus works on real-time PCR chemistry using two genes, the multicopy IS6110 and single-copy *nrdZ* gene for detecting *M. tuberculosis*.[14] It also offers detection of rifampicin resistance as a subsequent additional step.[15] To the best of our knowledge, other than a single study evaluating TruPlus on tuberculous meningitis (TBM),[16] there is no available literature on performance of TruPlus in diagnosing EPTB, including GITB. The current study was planned to evaluate the utility of TruPlus in diagnosing GITB and comparing its performance with two other NAATs, Xpert, and MPCR.

Materials and methods

Setting. Ileocecal biopsy specimens, collected by convenience sampling and processed in the Department of Medical Microbiology at PGIMER Chandigarh between 2011 and 2021, were included in this prospective case–control study. The study was approved by the Institute Ethics Committee. The specimens were divided into cases and controls on the basis of relevant microbiological and clinical details:

1 Cases:
   a Microbiologically confirmed GITB: cases that had acid-fast bacilli on smear or showed growth of *M. tuberculosis* on culture or both.
   b Clinically confirmed GITB: cases having no acid-fast bacilli and no growth on culture, but fulfilling the criteria of composite reference standard (CRS), as described previously.[17] As per the CRS, a case is labeled as clinically confirmed GITB if it fulfills at least one of the following: (a) isolation of *M. tuberculosis* from other site, pulmonary or extrapulmonary, (b) intraoperative or radiological features consistent with GITB, and (c) histopathology showing granulomatous inflammation but without caseation/acid-fast bacilli; along with favorable response to anti-tubercular therapy for patients falling under b and c.
2 Controls: patients with proven inflammatory bowel disease.

Microbiological processing and patient categorization. Following standard mycobacteriological precautions, each biopsy specimen was minced and homogenized thoroughly. The specimens were decontaminated using the NaACl-NaOH method. Initially, the specimens were subjected to Ziehl Neelsen smear and mycobacterial growth indicator tube (MGIT) culture (0.5 mL), and the remaining specimen was labeled and kept at −20°C. Depending upon smear, culture, and other clinical, radiological, and histopathological reports, the patients were grouped into microbiologically confirmed case, clinically confirmed case, and control. The most common symptoms were pain abdomen (70%), loss of weight (60%) and appetite (53%), vomiting (33%), fever (30%), diarrhea (10%), and partial obstruction (6.6%). Endoscopic findings like stricture, ulcerations and mucosal changes, and radiological findings like mesenteric lymphadenopathy, thickened peritoneum, and so forth were used in CRS. The specimens with sufficient volume (> 2 mL), amounting to 50 ileocecal biopsies (5 microbiologically confirmed GITB and 25 clinically confirmed GITB) were retrieved from −20°C at different times (ranging from 0 day [for prospectively collected biopsies] to 8 years [for archived biopsies]) for molecular testing. Due to financial constraints, 20 control specimens were randomly selected and subjected to three molecular tests each. Hence, the ratio of case : control was 3:2. The specimens were coded and randomly distributed to blind the investigator of the groups. Each specimen was divided into aliquots as follows: 0.5 mL for TruPlus, 1 mL for Xpert, and 0.4 mL for MPCR. Treatment was not withheld pending TruPlus results (Fig. 1).

TruPlus assay. A 0.5 mL of specimen was subjected to DNA extraction using Trueprep extraction protocol of TruPlus (Molbio Diagnostics, Verna, India).[14] A 6 μL of the extracted DNA was used for detecting *M. tuberculosis* while the remaining was stored at −20°C. *M. tuberculosis* was detected using in-built software on TruPlus (Molbio Diagnostics, Verna, India) based on real-time PCR. The amplification was completed in about 40 min, and the results were reported either as *M. tuberculosis* positive (MTB high, low and very low bacillary load) or negative. All those DNA that showed presence of *M. tuberculosis* were then subjected to Truenat RIF Dx reflex (Molbio Diagnostics, Verna, India) (TruRif) for detecting resistance to rifampicin.[15] Rifampicin susceptibility was reported as sensitive (RifS), resistant (RifR), and indeterminate (RifI).

Xpert assay. One milliliter of specimen was subjected to Xpert MTB RIF assay (Cepheid, Sunnyvale, CA, USA), following manufacturer's instructions. The results were interpreted as *M. tuberculosis* detected (bacterial load high, low or very low) or not detected. Rifampicin susceptibility was reported as sensitive, resistant, and indeterminate.

Multiplexed polymerase chain reaction. DNA was extracted using Qiagen Mini kit (Qiagen, GmbH, Germany), as per instructions provided by the manufacturer. MPCR using three genes, IS6110, MPB64, and protein B, (patented by Government of India [patent no. 340788 granted on July 8, 2020] for TB diagnosis), was performed as described previously.[10] For the validity of amplification, positive control (H37Rv strain of *M. tuberculosis*) and negative control (molecular grade water) were also subjected to amplification with each run. MPCR was

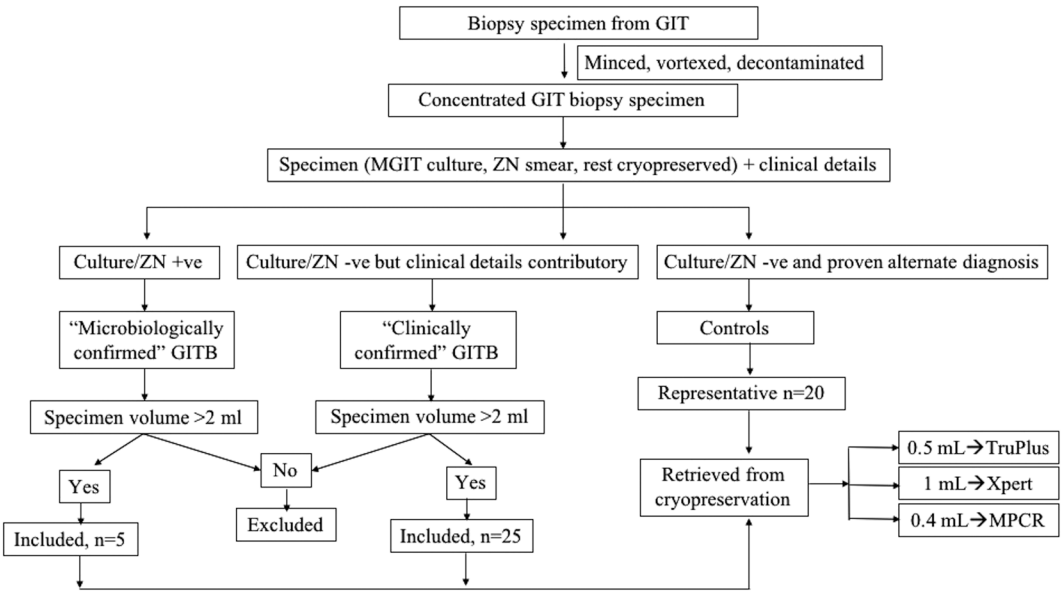

Figure 1 Diagrammatic representation of workflow and timing of different microbiological tests conducted on GIT biopsy specimens. GITB, gastrointestinal tuberculosis; MPCR, multiplexed PCR.

considered positive for GITB if any of the three genes were amplified.

rpoB gene sequencing. All isolates reported RifR and RifI and representative isolates reported RifS by TruPlus and Xpert were subjected to genotypic drug susceptibility testing (DST) using rpoB gene sequencing. rpoB gene sequencing was carried out on Big Dye Terminator 3.1 and ABI 3130 Sequencer (Applied Biosystems, Foster City, CA, USA).

Reference standards. The performance of TruPlus, Xpert, and MPCR in diagnosing GITB was evaluated using two reference standards. Culture was used as the reference standard for microbiologically confirmed GITB cases and CRS was used as reference standard for clinically confirmed cases. CRS consisted of clinical features suggested of GITB, microbiological evidence in the form of smear and culture, and radiological features of GITB, as described previously.[17]

For evaluating rifampicin resistance, both genotypic and phenotypic DST were used as the reference standards. Phenotypic DST was performed for all culture-positive cases. For culture-negative cases, rpoB gene sequencing was used as the reference standard for determining rifampicin resistance.

Statistical analysis. The performance was adjudged using sensitivity, specificity, positive, and negative predictive value, computed using standard formulae and expressed with 95% confidence interval. Categorical data were compared using χ^2 and Fisher's exact test. The performance of two tests was compared using McNemar test. Significance was attached to a P value < 0.05. The performance of the tests was also vetted against CRS as reference standard using Cohen's κ agreement, interpreted as 0.1–0.20—slight agreement; 0.21–0.40—fair agreement; 0.41–0.60—moderate agreement; 0.61–0.80—substantial agreement; 0.81–1.0—almost perfect agreement.

Results

Performance of different microbiological tests in diagnosing GITB. The overall sensitivity of smear, culture, Xpert, TruPlus, and MPCR in diagnosing GITB was 6.67%, 16.67%, 30%, 70%, and 83.33%, respectively (Table 1). None of the specimens from control group were reported positive by any of the tests, giving a specificity of 100% for all.

Performance of Xpert, TruPlus, and MPCR in diagnosing confirmed GITB. Among the five microbiologically confirmed cases of GITB, Xpert, TruPlus, and MPCR detected two, three, and four cases, respectively, producing a sensitivity of 40%, 60%, and 80%, respectively, against culture as the reference standard. MPCR additionally detected one case that was missed by both Xpert and TruPlus. One culture-positive case was missed by all the three NAATs. The specificity for all the three NAATs was 100%.

Performance of Xpert, TruPlus, and MPCR in diagnosing clinically confirmed GITB. Out of 25 clinically confirmed cases of GITB, Xpert, TruPlus, and MPCR detected 7, 18, and 21 cases, respectively, producing a sensitivity of 28%, 72%, and 84%, respectively, against CRS as the reference standard. All cases detected by Xpert were also detected by TruPlus and MPCR, and all cases detected by TruPlus were also detected by MPCR. Additionally, MPCR detected three cases that were missed by Xpert, TruPlus, and MGIT culture. The specificity of the three NAATs was 100% for clinically confirmed GITB cases.

Table 1 Evaluation of Xpert MTB/RIF (Xpert), Truenat MTB plus, and multiplexed PCR for diagnosis of GITB

Group	*N*	Smear+	Culture+	Xpert				Truenat MTB plus				MPCR
				N (%)	RIF (S)	RIF (R)	RIF (I)	*N* (%)	RIF (S)	RIF (R)	RIF (I)	*N* (%)
Microbiologically confirmed GITB cases	5	2 (40%)	5 (100%)	2 (40%)	1 (50%)	1 (50%)	—	3 (60%)	2 (66.66%)	1 (33.3%)	1 (33.33%)	4 (80%)
Clinically confirmed GITB cases	25	—	—	7 (28%)	2 (28.57%)	2 (28.57%)	3 (42.88%)	18 (72%)	6 (33.3%)	2 (11.11%)	10 (55.38%)	21 (84%)
Total	30	2 (6.66%)	5 (16.66%)	9 (30%)	3 (33.33%)	3 (33.33%)	3 (33.33%)	21 (70%)	8 (38.09%)	3 (14.28%)	11 (52.38%)	25 (83.33%)
Control group	20	—	—	—	—	—	—	—	—	—	—	—

GITB, gastrointestinal tuberculosis; MPCR, multiplexed PCR.

Comparative performance of the different tests in diagnosing GITB. The diagnostic yield of TruPlus was significantly higher than Xpert ($P = 0.001$) and that of MPCR was also was significantly higher than Xpert ($P = 0.0002$) (Table 2). The performance of TruPlus and MPCR was comparable ($P = 0.13$). Both TruPlus and MPCR showed moderate agreement ($\kappa = 0.65–0.8$) against CRS as the reference standard and Xpert showed fair agreement ($\kappa = 0.25$).

Among the four tests, TruPlus, Xpert, MPCR, and MGIT, 2/30 (6.67%) specimens were positive by all four tests and 7/30 (23.33%) other specimens were positive by all the three NAATs (Fig. 2). Eleven out of thirty (36.67%) specimens were detected by both TruPlus and MPCR and missed by Xpert. There were three specimens detected only by MPCR and one detected only by MGIT.

Performance of TruPlus and Xpert in detecting rifampicin susceptibility. Out of the 21 cases diagnosed as GITB by TruPlus, 8 (38.09%) were reported RifS, 3 (14.28%) were reported RifR, and the remaining 10 (47.62%) were reported RifI by TruRif (Table 3). All 10 cases reported RifI by TruPlus reported "MTB very low" load. Out of nine cases diagnosed as GITB by Xpert, three (33.33%) were reported as RifS, RifR, and RifI. Three cases reported RifI had "very low" bacterial load. The same three cases were reported RifR by both TruPlus and Xpert. Out of these three RifR cases, one was culture-positive, and two were culture-negative. The RifR in one culture-positive case was confirmed by phenotypic DST.

The reporting of RifR by both TruPlus and Xpert was concordant with rpoB gene sequencing. The three cases reported RifR had mutations at codon 531 ($n = 2$) and 526 ($n = 1$). All other cases reported RifS ($n = 8$) and RifI ($n = 10$) by TruRif did not have any mutation in the rpoB gene. The cases reported RifS ($n = 3$) and RifI ($n = 3$) by Xpert also had no mutations in rpoB gene on sequencing.

Analysis of turn-around time and estimated cost. The turn-around time for TruPlus (including DNA extraction, amplification, and detection of rifampicin resistance) was 150 min. The turn-around times for Xpert and MPCR were 60 min and 90, respectively. The average cost per sample (including reagents and consumables; excluding manpower, equipment, and electricity) was calculated to be $12 for TruPlus, $20 for TruRif, $22 for Xpert, and $10 for MPCR.

Discussion

The accurate diagnosis of GITB remains a challenge. This is because GITB has a very close mimicker; the Crohn's disease (CD). The two are misdiagnosed as each other in 50–70% cases.[18] The increasing burden of inflammatory bowel disease including CD in a country like India,[8] which is already endemic for TB including GITB and is witnessing increase in multidrug-resistant TB cases, this diagnostic chaos needs definite settling. Despite several efforts over the last two decades by the gastroenterologists, pathologists, radiologists, as well as microbiologists, the absolute differentiation between GITB and CD remains enigmatic. Kedia and Ahuja[19] opined that the key driver for this failure was lack

Table 2 Diagnostic performance of Truenat MTB Plus, Xpert, and MPCR against clinical reference standard for GITB

	Truenat MTB Plus	Xpert	MPCR
Positive/Total	21/30	9/30	25/30
Sensitivity [95% CI]	70.00% [50.60% to 85.27%]	30.00% [14.73% to 49.40%]	83.33% [65.28% to 94.36%]
Specificity [95% CI]	100% [83.16% to 100.00%]	100% [83.16% to 100.00%]	100% [83.16% to 100.00%]
PPV [95% CI]	100%	100%	100%
NPV [95% CI]	68.97% [56.26% to 79.33%]	48.78% [42.97% to 54.62%]	80.00% [64.25% to 89.90%]
P value	0.0001	0.007	0.0001
$\kappa^{\dagger}$	0.65	0.25	0.80
McNemars	Truenat MTB Plus vs Xpert - 0.0015 Xpert vs MPCR - 0.0002 Truenat MTB Plus vs MPCR – 0.13		

[†]0.1–0.20—slight agreement; 0.21–0.40—fair agreement; 0.41–0.60—moderate agreement; 0.61–0.80—substantial agreement; 0.81–1.0—almost perfect agreement.

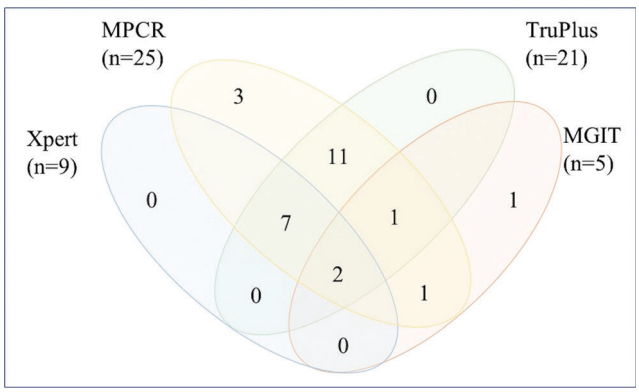

Figure 2 Venn diagram showing number of GITB specimens positive by each test. GITB, gastrointestinal tuberculosis; MPCR, multiplexed PCR.

of diagnostic armamentarium that can accurately identify the paucibacillary GITB as all available microbiological tests, including Xpert, lack sensitivity. This poor sensitivity, many a times, leads to unnecessary initiation of ATT or immunosuppressive therapy that is counter-productive for alternate diagnosis.[19] The present study evaluated diagnostic potential of TruPlus for GITB and if TruPlus could be the answer to the "TB-CD" diagnostic conundrum.

The sensitivity of TruPlus in diagnosing GITB from ileocaecal specimens was 70% in the current study, and none of the specimens from control group, consisting of patients with inflammatory bowel disease including CD, were reported positive. The evaluation of TruPlus on PTB[20] and TBM[16] produced a sensitivity of 91% and 78.7%, respectively, in prior studies, but there is no study reporting use of TruPlus for GITB diagnosis in available literature. The consolidated guidelines for rapid diagnostics of TB, provided by the World Health Organization in June 2020,[21] recommended TruPlus for diagnosing PTB and rifampicin resistance and recommended Xpert for diagnosing EPTB as well. However, it is pertinent to note that this recommendation was in part due to lack of sufficient literature on performance of TruPlus on EPTB samples. The performance of TruPlus was significantly better than Xpert (70% *vs* 30%) in the present study. Further, TruPlus detected additional 11 cases of clinically confirmed GITB that were missed by Xpert. These "clinically confirmed GITB" cases are the closest mimickers of CD and require accurate differentiation from it.

The role of Xpert in diagnosing GITB needs careful understanding. Although a meta-analysis of all studies before 2015 evaluating Xpert on EPTB specimens by Penz *et al.*[22] reported a sensitivity and specificity of 86% and 98%, respectively from five studies using GI specimens, it was acknowledged that the meta-analysis suffered from threshold effect and that the type and site of GITB sample were not taken into consideration. To explore the true potential of Xpert in diagnosing GITB, we did a PubMed search and analyzed all studies after Penz *et al.*, summarized in Table 4. The table shows that three types of samples have been subjected

Table 3 rpoB gene sequencing results of Xpert MTB/RIF, Truenat MTB plus, and MPCR positive cases

Test (number of positive cases)	RIF sensitive	RIF resistant	RIF indeterminate	rpoB gene sequencing RIF sensitive number (%)	rpoB gene sequencing RIF resistant number (%)	Codon at which mutations were observed
Truenat Mtb Plus (21)	8 (38.09%)	3 (14.28%)	10 (47.62%)	18 (85.71%)	3 (14.28%)	531 (2) 526 (1)
Xpert MTB/RIF (9)	3 (33.33%)	3 (33.33%)	3 (33.33%)	6 (66.66%)	3 (33.33%)	531 (2) 526 (1)
MPCR (25*)				15	3	531 (2) 526 (1)

Out of 25* MPCR positive cases, rpoB gene sequencing was carried out in 3 resistant cases and 15 representative sensitive cases.

Table 4 Analysis of studies evaluating performance of Xpert for the diagnosis of gastrointestinal tuberculosis showing the sensitivity of Xpert in each study and cumulative sensitivity of different sample types—fluid, stool, and biopsy/tissue

Sample type	S. no.	Author and year	Country	Total samples tested by Xpert	Samples positive by Xpert	Sensitivity against CRS	Rif resistance, *n*
Ascitic/peritoneal fluid	1	Liu *et al.*, 2019[23]	China	115	21	18.3%	0
	2	Ahmad *et al.*, 2018[24]	Pakistan	21	6	28.5%	0
	3	Rufai *et al.*, 2017[25]	India	67	12	17.9%	0
	Cumulative performance on fluid samples			282	33	11.7%	0
Stool	4	Talib *et al.*, 2019[26]	Pakistan	100	20	20%	0
Intestinal or peritoneal biopsy/tissue	5	Fei *et al.*, 2021[27]	China	42	14	33.3%	0
	6	Lowbridge *et al.*, 2020[28]	Malaysia	42	24	57.1%	3 (1 false RifR)
	7	Udgirkar *et al.*, 2019[29]	India	176	35	26.1%	0
	8	Dahale *et al.*, 2019[30]	India	28	17	60.7%	2
	9	Bellam *et al.*, 2019[31]	India	25	8	32%	0
	10	Kumar *et al.*, 2017[32]	India	37	3	8.1%	0
	11	Polepole *et al.*, 2017[33]	Zambia	8	2	25%	0
	12	Current study	India	30	9	30%	3
	Cumulative performance on biopsy samples			388	112	28.8%	8 (1 false RifR)

CRS, clinical reference standard; *n*, number; R, resistance; Rif, rifampicin; Xpert, GeneXpert.

to Xpert analysis for GITB—ascitic/peritoneal fluid, stool, and intestinal biopsy/tissue. The sensitivity of detection varied with the sample type; the cumulative sensitivity being 11.7% for fluids,[23–25] 20% for a single study on stool samples,[26] and 28.8% for biopsy/tissue samples[27–33] (inclusive of the present study). Thus, in all studies, subsequent to Penz *et al.*,[22] more uniform sample types were methodically subjected to Xpert, and the sensitivity was found to range between 8% and 60.7%. The sensitivity of 30% by Xpert in the present study is in tune with the cumulative of 28.8% reported for biopsy/tissue specimens. The ileocecal junction is the most favored site of GITB, being involved in nearly 64% cases,[6] due to minimal activity, greater absorption, and neutralized digestive juices. The lymphoid aggregates at this site also yield better bacillary load for *M. tuberculosis*.[34] These could be the reasons for higher sensitivity in biopsy/tissue specimens than fluids. For the same reason, ileocecal biopsy was used in the present study.

Multiplexed polymerase chain reaction detected 83.3% cases of GITB in the present study, producing higher sensitivity than all other tests including TruPlus. This could be attributed to incorporation of three different genes that aided in detecting even those cases that lacked one or two of the three genes. IS6110 has been reported to be absent or present only as single copy in 10–40% of north Indian population[35] while MPB64 tends to miss other members of *M. tuberculosis* complex like *Mycobacterium bovis*.[36] This findings is similar to previous studies where the sensitivity of MPCR was reported as 87% for GITB samples.[10] Even in other EPTB sample like cerebrospinal fluid, MPCR has reported a higher sensitivity than Xpert and Xpert Ultra earlier.[37]

It was interesting to note that there was one GITB case that was positive by MGIT and was missed by all the three molecular tests, TruPlus, Xpert, and MPCR. Possibly, this strain of *M. tuberculosis* complex lacked sufficient number of genes targeted by these NAATs. Previous researchers have also reported similar finding on TBM samples.[37–39]

In determining rifampicin resistance, the performance of TruRif and Xpert was similar, in the current study. The three RifR cases, as confirmed by rpoB gene sequencing, were correctly identified by both TruRif and Xpert. A higher number of RifS cases were detected by TruRif than Xpert (8 *vs* 3). But TruRif also reported a higher number of RifI cases than Xpert (10 *vs* 3). Because the reporting of rifampicin susceptibility is based on the bacillary load, paucibacillary nature of GITB could have resulted in "indeterminate" reporting by TruRif, as all cases reported RifI had "very low" bacterial load. The reporting of RifR and RifS was 100% concordant with rpoB gene sequencing in the present study for both TruRif and Xpert. Previous studies have, however, reported false-RifR and false-RifS with both TruRif[16] and Xpert[28,40] on other EPTB samples. More prospective studies evaluating TruRif on GITB as well as other EPTB samples are required and till then the rifampicin susceptibility should be confirmed with phenotypic or genotypic DST.

TruPlus, with its higher sensitivity and easier portability than Xpert, could prove to be a useful tool for diagnosing TB and its various forms at the primary health-care level in endemic countries like India. The Global TB report stated that out of 10 million TB cases in 2019, 2.9 million (29%) were not detected/reported to national programs.[3] An important factor behind these "missed" cases could be the inherent limitations of the available diagnostic armamentarium to reach these people living in areas with poor connectivity and resources. TruRif was also reported to successfully identify $>$ 90% mutations associated with rifampicin resistance globally.[41] Thus, TruPlus could help in bringing about the required change for TB elimination efforts by identifying those "missing" and "resistant" cases.

Although both TruPlus and Xpert are made available free-of-cost by the Government of India, our analysis show that cost of diagnosing GITB alone by TruPlus was $12 and that for identifying RifR was $20. Hence, the total cost of diagnosing GITB with RifR was $32 with TruPlus. This was higher than the total cost of Xpert that detected both the things simultaneously at $22. In a cost–benefit analysis by Lee *et al.*,[42] Truenat was found to have improved linkage-to-care and was cost-effective in the public sector health care of India as a point-of-care test. Further, 70% of the technical staff reported it to be similar or technically less demanding than Xpert.[41] In contrast to these two assays, the cost per

sample for MPCR was observed to be much cheaper ($10) in the present study; however, it is limited to detection of *M. tuberculosis* only, without rifampicin susceptibility and requires more hands-on techniques.

It is important to note that although TruPlus fared well in diagnosing GITB in the current study, its negative predictive value was substantially low (69% in the current study). This means that TruPlus, just like Xpert, can only be used for ruling-in GITB, but a negative result cannot rule-out GITB. Hence, TruPlus could also serve as one of the first tests for GITB, but definitely not the last.

The study has following limitations. The sample size studied was small, 30 cases and 20 controls. This was because only specimens with adequate volumes could be subjected to all microbiological investigations targeted in the study. Financial implications limited the control group to 20 cases. The molecular tests were conducted on frozen specimens. Although the yield of *M. tuberculosis* DNA is not altered in properly cryopreserved specimens,[43] future studies may evaluate larger cohorts of fresh GI biopsy specimens also. TruPlus has the limitation of separate steps for DNA extraction, *M. tuberculosis* detection, and rifampicin resistance reporting. Further, it also reports only rifampicin resistance.

Looking at the pros of TruPlus, the three-step procedure allows availability of extracted DNA for future processing and conserves the resources as only those samples are subjected to rifampicin resistance detection in which *M. tuberculosis* has been identified. Future models could incorporate multiplexing to simultaneously detect *M. tuberculosis* and presence of resistance to various drugs.

The study concludes that TruPlus surpassed the sensitivity of Xpert in diagnosing GITB and can be used as an important tool for accurate diagnosis of GITB even in remote areas of the country with minimal facilities and minimally trained staff. The detection of RifR by TruRif was concordant with Xpert and rpoB gene sequencing; however, it reported higher number of cases as RifI.

Acknowledgments

None.

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