International Journal of Mycobacteriology 3 (2014) 205–210

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Evaluation of the Indian TrueNAT micro RT-PCR device with GeneXpert for case detection of pulmonary tuberculosis

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ARTICLE INFO

Article history:
Received 13 February 2014
Received in revised form
26 April 2014
Accepted 30 April 2014
Available online 2 June 2014

Keywords:
RT PCR
Pulmonary tuberculosis
GeneXpert
TrueNAT MTB

ABSTRACT

To evaluate the performance of TrueNAT (RT Micro PCR device) assay in comparison with GeneXpert on sputum samples from pulmonary cases of tuberculosis. 274 samples were processed to detect MTB by ZN smear examination, MGIT culture and molecular methods that included RT-PCR (ABI 7500 & TrueNAT) and GeneXpert for case detection of TB. The overall performance of the test with MGIT(Mycobacterium Growth Indicator Tube) culture as gold standard, sensitivity of smear, RT PCR/TrueNAT and Genexpert was 61.5% (CI:53.3–69.3%), 94.7% (CI:89.8–97.6%) & 96.0% (CI: 91.5–98.5%), respectively. Amongst the S+ (108) samples, RT-PCR/TrueNAT and GeneXpert showed a sensitivity of 99% (CI:94.9%–99.8%) and 100% (98.6%–100.0%), respectively. High concordance was observed between GeneXpert and TrueNAT for case detection of TB. The GeneXpert MTB/RIF test was independent on the user's skills. It has a short turn-around time and simultaneously detects RIF resistance with M. tuberculosis in less than 3 h. The TrueNAT MTB has good sensitivity and specificity in case detection with hands on time of less than 3 h as well as fits the requirements in resourcelimited health care settings. Larger, multi-site studies are required to obtain better estimates of the performance of TrueNAT MTB.

Background

Diagnosis of tuberculosis (TB) in the developing world presents an urgent need for novel solutions. Despite effective anti-TB medication, TB continues to contribute to the large death toll caused by curable infectious diseases. The global annual incidence estimates about 8.8 million cases, of which 1.5 million cases are from India [1]. Since 2007, the World Health Organization (WHO) has approved many new diagnostic tests for TB [2,3]. However, smear microscopy, which has varying sensitivity [4] and under-reports a large number of early stage cases, is still the most widely used test in the developing world.

The commercially available automated, liquid MGIT (Mycobacterium Growth Indicator tube) culture system is time-consuming and requires specialized laboratories. Molecular tests such as polymerase chain reaction (PCR), though sensitive, still take time as specimens are often sent to distant

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http://dx.doi.org/10.1016/j.ijmyco.2014.04.003

referral laboratories. The expense and infrastructure involved in PCR testing establishes a barrier to implementation in most of the TB-endemic countries. Quick and affordable diagnoses are critical to prevent TB-related casualties.

There has been substantial interest in developing cost-effective molecular tests that can be used "near-patient" as a means to curb the TB menace. With combined advantages of affordability, ease of use, diagnostic sensitivity and portability, low-cost, point-of-care molecular devices that enhance the efforts to treat diseases before they spread and cause irreversible damage to the patient's health are good candidates for wide-scale use among the peripheral laboratories in India and other countries of South-East Asia, which accounts for 50% of the global burden of TB.

A recent example of the developments in the field of TB diagnostics is the GeneXpert system (Cepheid, Sunnyvale, CA), an automated real-time PCR system that simultaneously detects both *Mycobacterium tuberculosis* complex (MTBC) and Rifampicin (RIF) resistance in less than 2 h. Recent studies reported high sensitivity and specificity of the Xpert MTB/RIF test in the detection of TB from respiratory specimens [5–9], collected from patients living in countries with both high and low prevalence of TB. GeneXpert is endorsed by the WHO for use in national TB control programs. In endemic countries, the use of the Xpert MTB/RIF is often limited to laboratories with a controlled environment [10,11].

There is an urgent need for cost-effective molecular tests that can be used as point-of-care to curb the TB menace. Tests that can be used in "low-infrastructure" settings could save thousands of lives that are otherwise lost to TB every year [12]. Bigtec Laboratories, Bangalore, India has developed a battery-operated, portable micro PCR device, the TrueNAT MTB, RT-PCR micro device, as well as Trueprep MAG for extraction of DNA directly from samples for early detection of TB.

In a pilot study [13], it was reported that a novel TB test, the TrueNAT MTB, was able to detect TB rapidly with good sensitivity in comparison with a Composite Reference Standard (CRS). The test, TrueNAT (Fig. 1), offered faster and accurate results as compared with in-house nested PCR protocol. Using a CRS as the benchmark, a sensitivity and specificity of 91.1% and 100%, respectively, was reported for TrueNAT. In the current study, its performance was assessed against the widely accepted and WHO approved GeneXpert MTB/RIF for case detection of TB.

Fig. 1 – TrueNAT micro PCR device for chip-based real-time PCR.

Methods

Ethics

This study was approved by the Institutional Review Board (IRB). Waiver of consent was obtained by the IRB, as the study was carried out on left-over banked specimens identified by a laboratory generated number with no traceability to the patients. The TrueNAT MTB/GeneXpert results were not used in clinical decision making.

Settings

Sample collection, Smear Microscopy, MGIT culture and GeneXpert was performed at Hinduja Hospital and Medical Research Centre, Mumbai. The TB lab is accredited with the College of American Pathologist (CAP), National Accreditation Board for Laboratories (NABL) and Central TB Division, Governmet of India (CTD, GOI) for liquid culture and DST. It is the referral laboratory for TB. Real-time PCR and TrueNAT MTB tests were performed by trained Hinduja hospital staff at Bigtec Labs, Bangalore.

Study population and specimens

This was a blinded study to determine the performance of the TrueNAT in patients with symptoms of pulmonary TB in comparison with conventional methodologies (smear and culture) and GeneXpert. Sputum specimens were collected from patients presenting routinely to hospital with suspected pulmonary TB. Standard diagnostic follow-up (smear, culture, GeneXpert) was performed on all patients. Left-over sputum specimens were tested using TrueNAT. A total of 274 ($n = 274$) sputum samples were collected from patients suspected of having TB (Fig. 3).

Laboratory work-up of sputum specimens

Ziehl Neelsen (ZN) smear: Direct and concentrated acid-fast bacillus (AFB) microscopy (ZN staining) was performed and specimens were graded as per WHO recommended criteria, followed by sputum processing with 2% N-acetyl-L-cysteine and sodium hydroxide (NALC–NaOH) and centrifugation [14,15]. The re-suspended pellet was subjected to cultivation on liquid medium MGIT, supplied by Becton Dickinson.

Digested and decontaminated (2% NALC–NaOH) sputum specimens that were culture negative for mycobacterium and confirmed "Non-TB" by sequencing were pooled for use as a negative control. A suspension of *M. tuberculosis* H37RV was prepared in sterile saline and adjusted to the density of a 1.0 McFarland standard. The suspension was diluted 1:10 in saline and used to spike the pooled above-mentioned negative control and used as a positive control. Spiked specimens were stored at −70 °C until further processing.

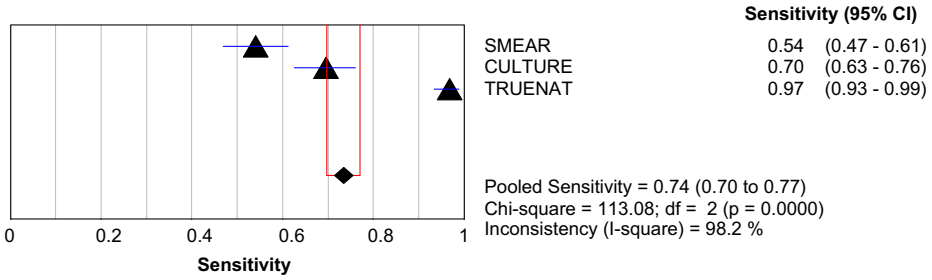

Fig. 2 – Forest plot for sensitivity values of Smear, Culture, and True NAT (microbiological and molecular methods) with pooled sensitivity as compared with GeneXpert. Performance of molecular methods studies reporting sensitivity. Point estimates of sensitivity estimates from each study are shown as solid circles. Solid lines represent the 95%CI (CI = confidence interval).

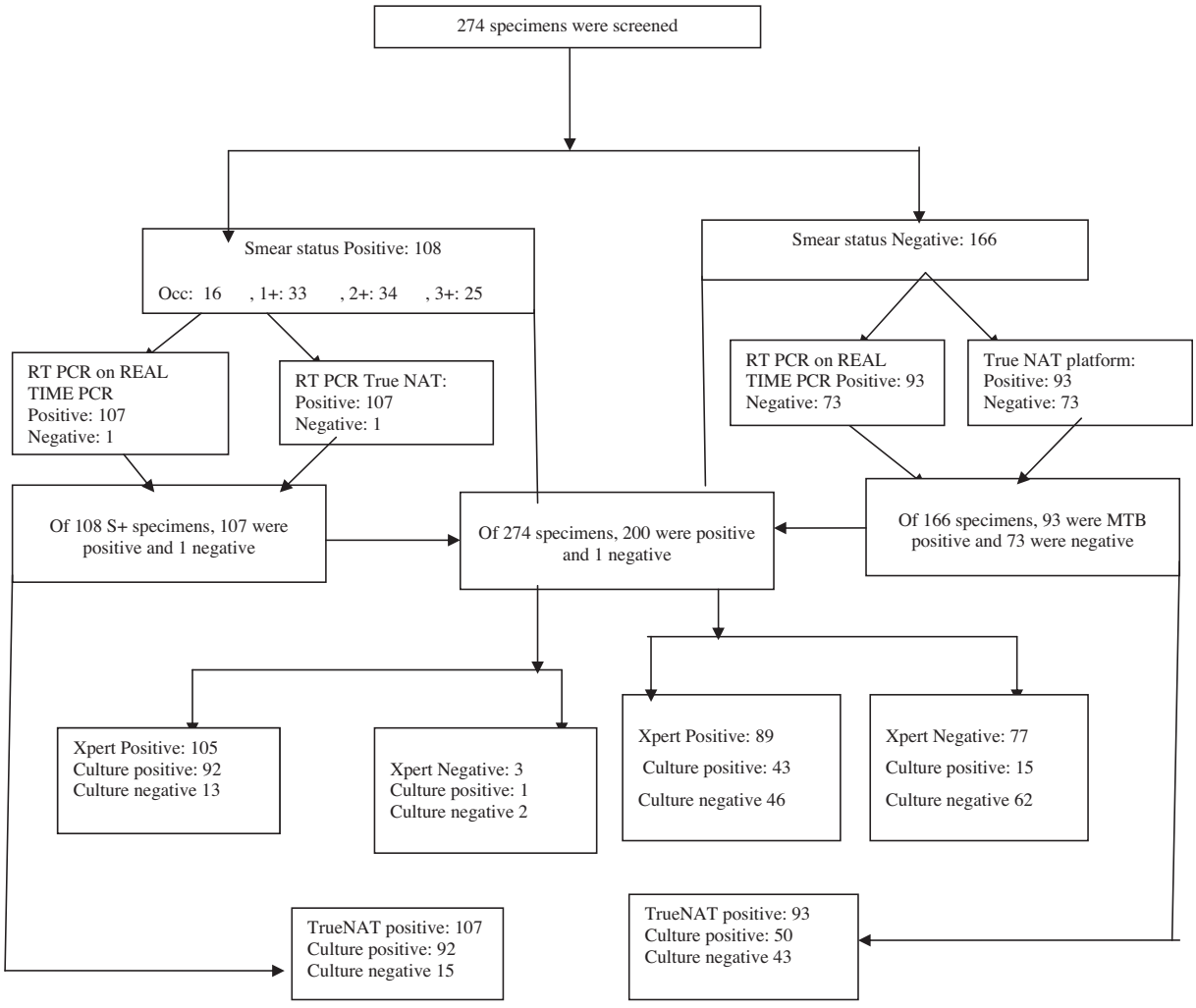

Fig. 3 – Flow diagram of study selection.

TrueNAT MTB test

DNA extraction using Trueprep-MAG protocol

Untreated sputum specimens were processed as per manufacturer's instructions of Trueprep-MAG Sputum kit with a starting volume of 500 μl being added to the sample pre-treatment tube [13].

Real-time PCR on chip

5 μl of DNA extracted added to the TrueNAT MTB microchip containing lyophilized mastermix and the real-time PCR was done using a pre-programmed profile on the device. Results were observed on the screen. The lyophilized mastermix included proprietary primers and a probe specific to the *M. tuberculosis*.

Table 1 – Performance (% of cases detected) of molecular tests in various specimen categories.

Test	S+ (n = 108)	C+ (n = 151)	S+C+ (n = 93)	S−C+ (n = 58)
Xpert MTB/RIF	100 [96.5–100.00]	96.02 [89.09–98.63]	100 [96.5–100.00]	90.14 [88.71–94.35]
TrueNAT MTB	99.07 [94.2–99.95]	92.71 [88.65–97.06]	98.92 [94.2–99.95]	86.21 [74.07–93.44]

Table 2 – Comparison of TrueNAT MTB with Xpert MTB/RIF results.

(n = 274)	GeneXpert		
		Positive	Negative
TrueNAT MTB	Positive	198	3
	Negative	8	65

Real-time PCR on ABI 7500

PCR reactions were run using the DNA extracted using the Trueprep-MAG protocol. 4 μl of extracted DNA was mixed with 6 μl of the TrueNAT MTB mastermix and real-time PCR was performed on real-time PCR (Applied Biosystems) under the following cycling conditions: 1 min at 95 °C and 45 cycles of 10 s at 95 °C and 34 s at 58 °C.

Buffers, reagents and mastermixes

All buffers and reagents used for nucleic acid extraction and all mastermixes used for PCR are proprietary constituents of the Trueprep-MAG Sputum and TrueNAT MTB kit.

Xpert MTB/RIF

The assay was performed as per the manufacturer's instructions [5].

Statistical analysis

Evaluation of the TrueNAT MTB test was performed in comparison with the other molecular methods for detection of *Mycobacterium tuberculosis* DNA from sputum, following the STARD recommendations [16]. Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value, were calculated by using the http://www.vassarstats.net/ and MedCalc software online clinical calculator.

Results

Evaluation of TrueNAT MTB was performed using the Xpert MTB/RIF as a benchmark, another well standardized molecular method. A total of 274 samples were screened, the Xpert MTB/RIF assay was repeated for 17 samples (9 had Error messages and 8 were Invalid). After repeating, 4 samples were still invalid, hence excluded from the study. Of the 4 invalid samples, 1 was S+C+, and the remaining 3 were S−C−. The inhibition rate of GeneXpert was 6.2% (17/274). Out of 274 samples, as shown in the flow chart (Fig. 3), 93/274 were S+C+, 58/274 were S−C+, 15/274 were S+C− and 108/274 were S−C−; 108/274 were smear positive (S+), 151/274 were culture positive (C+), 93/274 were smear and culture positive (S+C+). Among the 166/274 smear negative (S−) cases, 58 were culture positive (S−C+).

In S+C+ category, Xpert detected 93 (100%) samples, whereas TrueNAT MTB and RT-PCR (Applied Biology) detected 92 (98.9%) samples as MTB. In S−C+ category, Xpert detected 52 (90.1%) of 58 samples, whereas TrueNAT MTB detected 50 (86.8%) and RT-PCR (Applied Biology) detected 51 (87.9%) of 58 samples as MTB, as shown in Table 1.

Among the S+ (108) samples, TrueNAT MTB and GeneXpert detected 99.0% (CI: 94.9–99.8%) and 100% (96.5–100.00%) of sensitivity respectively. In the overall performance of the test keeping GeneXpert as the standard, the sensitivity of smear, culture and RT PCR/TrueNAT is as shown in Table 3 and Figure 2.

Sensitivity and specificity for smear-positive pulmonary samples is much higher as compared with the overall sensitivity for the remaining tests. GeneXpert and TrueNAT showed good sensitivity for S+ pulmonary samples. These sensitivities are analogous to previous studies [5,17].

The TrueNAT MTB results (which were run on both the TrueNAT micro PCR device and the RT-PCR) were largely concordant with Xpert MTB/RIF results (Tables 1 and 2). Out of 247 samples, 229 samples (92.7%) showed identical results (detected or undetected) for both the Xpert MTB/RIF and TrueNAT MTB systems. Of the 18 discordant results, 3 samples were positive by TrueNAT MTB, but negative by Xpert MTB/RIF. On the other hand, 8 samples were positive by GeneXpert, but negative by TrueNAT MTB. Of this group, all samples were S−C−. The TrueNAT MTB assay had a higher sensitivity when compared with conventional methodologies.

Discussion

In the current study, TrueNAT assay showed a high concordance with the GeneXpert system. Thus, the system might be a potential, accurate and rapid method for detecting TB cases in high TB burden countries like India.

Table 3 – Sensitivity, specificity, of TrueNAT MTB and GeneXpert MTB/RIF with smear and culture method as reference with test performance of TrueNAT with GeneXpert. All the figures are in %. Figures in the brackets indicate 95% confidence interval.

Test Performance	MGIT culture	GeneXpert	ABI/TRUENAT
Vs smear			
Sensitivity	86.11% (78.13–92.01%)	100% (96.61–100%)	100% (96.61–100%)
Specificity	65.06% (57.29–72.28%)	40.96% (33.40–48.85%)	43.98% (36.29–51.88%)
Vs culture			
Test Performance	Smear	GeneXpert	ABI/TRUENAT
Sensitivity	61.59% (53.33–69.38%)	96.03% (91.55–98.52%)	94.70% (89.82–97.68%)
Specificity	87.80% (80.68–93.01%)	50.41% (41.25–59.54%)	52.85 % (43.64–61.91%)
Test Performance	GeneXpert vs TrueNAT		
Sensitivity	96.12% (92.49–98.30%)		
Specificity	95.59% (87.63–99.03%)		

Culturing concentrated specimens–the current gold standard–can detect very low concentrations of organisms. However, the current liquid and solid culture systems take several weeks to yield results. Also, the testing process requires sophisticated laboratory and specifically trained personnel. These technologies are therefore suited to centralized laboratories and are not suitable for use in peripheral settings. Although molecular amplification is already a proven technology in TB diagnosis, existing testing methods are not applicable in most of the TB endemic countries due to lack of an adequate healthcare infrastructure.

In a previous study, TrueNAT assay with culture and CRS as the gold standard was evaluated. While this is the most convenient reference method [18,19], the platform, which has been rolled out in various endemic countries, could potentially lead to millions of disability adjusted life years (DALYs) saved as faster treatment in response to a quicker diagnosis would ensure that the transmission of the disease is curtailed. However, the Xpert MTB/RIF requires a continuous supply of electricity to maintain a controlled environment to perform the test and hence, is not practical in near-patient settings. Patients/samples are still required to travel to the nearest testing center. This limits its use in peripheral settings and in active case finding (ACF) programs [18]. In routine practice, the MTB/RIF test is much faster (3–24 h) than culture, which requires 3–6 weeks. The sensitivity of the TrueNAT MTB is much higher than smear microscopy or culture (Table 3).

With the TrueNAT MTB test, specimens can be tested as soon as a patient presents with symptoms. The entire setup, being battery operated and portable, can be deployed at the lower levels of the healthcare pyramid. This can help in reducing the logistical hurdles involved with transporting sputum specimens to distant referral laboratories. The turnaround time between collection of sample and diagnosis of TB could be greatly reduced as well. Processing a single sample on each device decreases the cross-contamination issues. As a portable platform, it could also be utilized in ACF programs, which are the current need of the hour to improve detection rates and reduce the incidence of infection. There is a great need for rapid, accurate diagnostic products for early diagnosis of TB in low-prevalence and high-prevalence areas to prevent the spread of TB.

A limitation of TrueNAT MTB is that it cannot determine MDR-TB, which is of most significance in high burden countries like India. But, as compared with any other molecular method, it is very cost effective.

In conclusion, the GeneXpert MTB/RIF assay is independent of the user's skills, and routine staff with minimal training can use the test. It has a shorter turnaround time and simultaneously detects RIF resistance in less than 3 h. The TrueNAT MTB test has good sensitivity and specificity for case detection of TB as compared with Xpert with hands-on time less than 3 h. However, larger, multi-site studies are required to obtain better estimates of the performance of TrueNAT MTB.

Conflict of interest

None declared.

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