

SHORT COMMUNICATION

EasyNAT MTC Assay: a Rapid Method to Detect Mycobacterium Tuberculosis in Respiratory Specimens

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SUMMARY

Background: This study aimed to effectively evaluate the diagnostic performance of the EasyNAT *Mycobacterium tuberculosis* complex (MTC) assay for tuberculosis (TB) detection from sputum.

Methods: The retrospectively analyzed data was collected from September 1, 2021, to November 1, 2023, in our hospital.

Results: Forty EasyNAT-positive sputum specimens were simultaneously detected using the GeneXpert MTB/rifampicin (RIF) assay. The concordance rate between the EasyNAT and GeneXpert MTB/RIF assays was 100%.

Conclusions: Because of the complexity of detecting RIF resistance data information, the rapid EasyNAT system used in conjunction with GeneXpert might be a better choice for the detection of TB in hospitals.

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KEYWORDS

EasyNAT system, GeneXpert MTB/RIF, TB, sputum, PCR, time threshold

INTRODUCTION

Compared with the large decline observed in 2020, an estimated 10.6 million people were falling ill with TB in 2021 (45% and 23% of them were in Southeast Asia and Africa, respectively) [1]. We should apply the lessons learned in overcoming the COVID-19 pandemic to the management of TB. In addition to the detection technologies reviewed by the World Health Organization (commercial liquid culture and rapid speciation strip test, LED microscopy, GeneXpert MTB/RIF, and urine LAM rapid test), loop-mediated amplification (LAMP) techniques have been important in advancing pathogen detection since 2016 [2]. Accurate and rapid diagnosis is critical for screening active TB, and reporting results are usually available within several hours. However, the costs associated with GeneXpert MTB/RIF are a barrier to its widespread use in resource-limited settings; therefore, a novel, user-friendly, rapid, robust, sensitive, specific, and affordable diagnostic tool for detecting TB is urgently needed. The COVID-19

pandemic offered the opportunity to increase the levels of TB testing. A commercial molecular assay based on LAMP was reported as a potential tool for the detection of TB in children [3]. In this study, we evaluated the CPA-based, innovative EasyNAT system developed by Ustar Biotechnologies Co., Ltd., for the detection of TB from sputum samples.

MATERIALS AND METHODS

The GeneXpert/RIF system reports a semiquantitative estimate of the TB concentration (very low, low, medium, high) and RIF resistance information from sputum samples. The sputum specimens are liquefied using GeneXpert MTB/RIF sample reagent and incubated for approximately 15 minutes, then 2 mL of the mixture are dispensed into a GeneXpert MTB/RIF cartridge before being loaded into a GeneXpert MTB/RIF instrument. The GeneXpert MTB instrument takes 1 hour 42 minutes to detect TB and determine the RIF resistance of the sputum specimens.

The EasyNAT MTC assay detects the TB insertion sequence 6110 but could not provide RIF resistance data. The DNA target sequence could be amplified at an appropriate constant temperature by multiple primers. According to the instructions, sputum is liquefied using 4% NaOH, then vortexed for 30 seconds, incubated at room temperature for 15 minutes, then 1 mL of the liquefied sample is centrifuged at 12,000 rpm for 3 minutes. The sediment and the MTC DNA extracting solution are then mixed prior to on-machine detection. The EasyNAT MTC assay comprises two detecting tubes: a left detecting tube and a right detecting tube. Regardless of the internal control value for the tubes, either one or both of the detecting tubes gives the time threshold (Tt) value, the result is a valid positive according to the instructions of the EasyNAT MTC assay. The EasyNAT instrument takes 1 hour 35 minutes to detect TB from sputum.

In the initial phase, the concordance rate between EasyNAT-negative and GeneXpert MTB-negative detection was 100% for 40 normal sputum samples tested as a part of performance verification for the EasyNAT assay. To obtain RIF resistance data for internal regulation in our hospital, the EasyNAT-positive specimens were simultaneously tested using the GeneXpert MTB/RIF assay for confirmation on the same sample.

Negative and positive controls were performed for each new batch number.

RESULTS

Of the 40 EasyNAT-positive samples detected between September 1, 2021, and November 1, 2023, all were also GeneXpert-positive, including 3 specimens with very low MTB concentrations, 16 specimens with low concentrations, 12 specimens with medium concentrations,

and 9 specimens with high concentrations. Among these, there were three RIF-resistant MTB specimens, two of which showed no detection at Probe B and one showed no detection at Probe E (data not shown).

As shown in Figure 1, both left and right detecting tubes showed Tt values for all of the specimens, with 21 GeneXpert-positive specimens showing medium/high MTB concentrations. However, seven specimens with detection at either the left or the right detecting tube showed Tt values when analyzing the 19 GeneXpert-positive specimens with very low/low MTB concentrations. Regardless of whether the MTB concentrations of GeneXpert MTB were low or high, the mean Tt values of the left detecting tube in the EasyNAT system were shorter than those of the right detecting tube.

DISCUSSION

Point-of-care diagnostic tests include smear microscopy, cartridge-based NAAT, chip-based NAAT, TB-LAMP, lateral flow assays, and portable digital chest X-rays [4]. Even though the early methodologies developed by Ustar Biotechnologies Co., Ltd., relied on observing visible bands on the test strips, such methods could be performed in clinical laboratories in developing countries with good specificity and sensitivity for the detection of TB [5]. It has been reported that the EasyNAT system is suitable as an initial testing step for *Mycoplasm*a pneumonia diagnosis, but is not suitable in isolation as an initial testing step for the diagnosis of TB lymphadenitis in children [6,7]. Such methods therefore require continuous improvement to increase their accuracy, rapidity, and ease-of-use. Furthermore, the commercial EasyNAT assay (an upgraded version of CPA with automatic interpretation) should be adequately evaluated and validated in clinical laboratories where it is intended for diagnostic use.

Compared with the EasyNAT system, the GeneXpert/RIF system requires a shorter sample processing time because centrifugation is not required for sputum specimens [8]. To obtain RIF resistance data for internal regulation in our hospital, the EasyNAT-positive specimens were tested again using the GeneXpert MTB/RIF assay. The concordance rate between EasyNAT-positive and GeneXpert MTB-positive detection was 100% for the samples tested in this retrospective study, and the proportion of RIF-resistant specimens was 7.5% (3/40). We also found that both left and right detecting tubes showed Tt values for the specimens with medium and high MTB concentrations, but the phenomenon appeared in only 12/19 specimens with very low and low MTB concentrations. This indicated that the design of the EasyNAT MTC assay (with two detecting tubes) successfully avoided false-negative results in low MTB concentration specimens in this study. These retrospective data could also be applied to device-to-device comparisons as part of the quality control and system management of medical laboratories. In addition, the con-

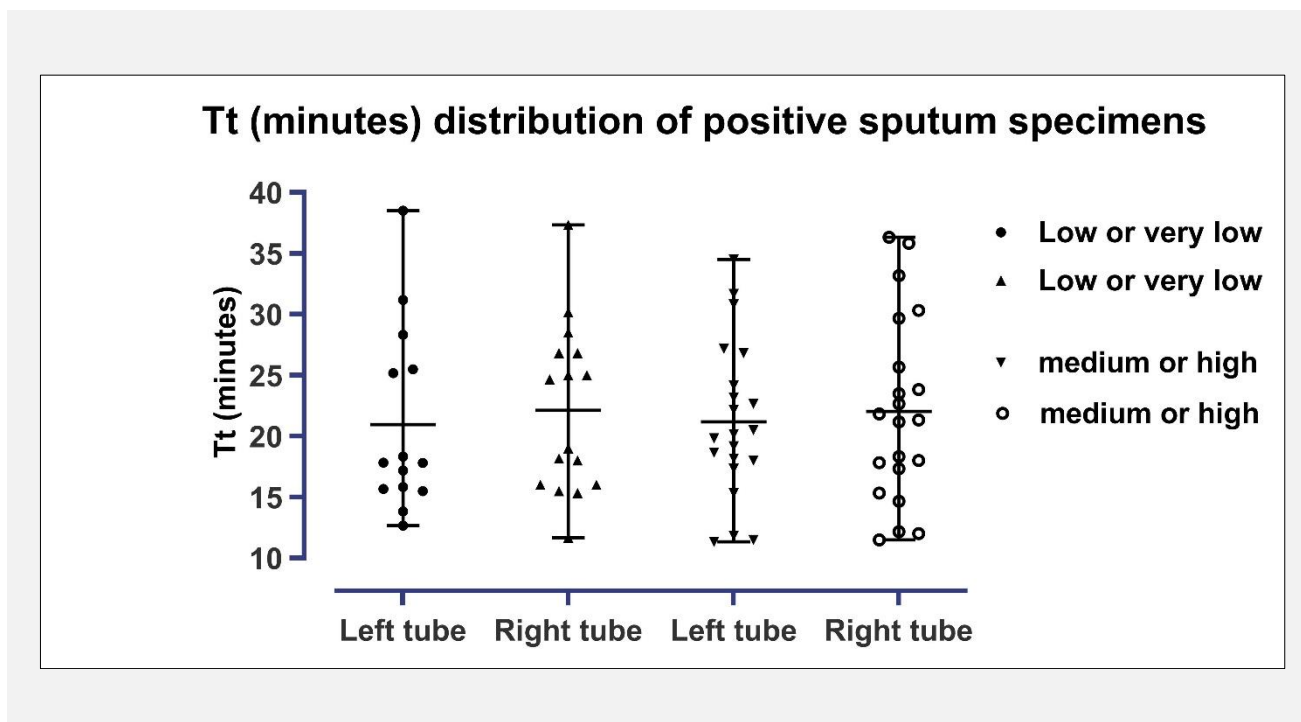


Figure 1. Compared with GeneXpert-positive samples (very low/low and medium/high MTB concentration), the Tt values (minutes) distribution of both tubes using the EasyNAT system are shown.

Tt - Time threshold.

cordance rate between EasyNAT-negative and Gene Xpert MTB-negative detection was also 100% in the initial phase. Considering the advantages and disadvantages of both instruments, the future integration of the EasyNAT system with GeneXpert may provide a new, effective tool for combatting TB.

The limitation of this study was the relatively small sample size. Our findings underscore the need for larger studies to evaluate the diagnostic performance of the EasyNAT MTC assay for TB detection in respiratory specimens in the future.

CONCLUSION

Our findings suggest that the EasyNAT system is suitable as an initial testing step for TB diagnosis from respiratory specimens. It might be the preferred choice for the detection of TB in clinical settings in conjunction with GeneXpert.

Declaration of Interest:

None.

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