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DIAGNOSING EXTRA-PULMONARY TUBERCULOSIS (EPTB) BY TRUENAT MTB/RIF TEST IN COMPARISON WITH MICROSCOPY AND CULTURE

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ABSTRACT

INTRODUCTION: A big issue in world health is tuberculosis. Mycobacterium tuberculosis is the causative agent of extra-pulmonary tuberculosis (EPTB), which may manifest in a variety of ways and locations. Lymph nodes, CNS, pleural, abdominal, pericardial, urogenital, cutaneous, ophthalmic, and other sites are all potential sites of EPTB. An innovative approach, Truenat is a battery-operated, microdevice based real-time polymerase chain reaction (RT-PCR) for detecting mycobacterium tuberculosis (MTB) in clinical samples. A second chip may be used as a follow-up test to determine if Truenat MTB 'detected' samples have rifampicin resistance.
The study's stated goals are to(1) investigate the current state of the art in EPTB diagnostic technology and(2) assess the specificity and sensitivity of these technologies.

MATERIALS AND METHODS: At IGIMS in Patna, Bihar, India, researchers conducted a cross-sectional study over the course of fifteen months. A total of 254 clinical samples from areas other than the lungs were examined. **Inclusion Criteria:** The trial included all patients with presumed EPTB who were at least one year old, had a prior history of anti-tubercular therapy (ATT), and whose treatment had failed during their most recent session of treatment. **Exclusion Criteria:** Participants who were on ATT or had pulmonary TB were not included in the research. Ziehl-Neelsen (ZN) staining was used for smear microscopy, Mycobacterium growth indicator tube (MGIT) culture, and Truenat PCR for Mycobacterium tuberculosis (MTB). The data was presented using summary statistics with a 95% Confidence Interval (CI), and comparisons were conducted between the tests.

RESULT: A total of 254 samples were collected from areas beyond the lungs. Truenat found 26 cases of tuberculosis out of 254.

CONCLUSION: For the diagnosis of EPTB in areas with limited resources, the Truenat MTB test provides an efficient and quick molecular diagnostic tool.

Keyword: Mycobacterium tuberculosis, Extra-pulmonary tuberculosis, chip-based.

INTRODUCTION:

A big issue in world health is tuberculosis. One of the leading killers on a global scale is tuberculosis. About 2.7 million cases of tuberculosis are reported from India each year, out of an estimated 10.0 million cases globally [1]. EPTB, which is caused by MTB, affects many organs or locations. Lymph nodes, CNS, pleural, abdominal, pericardial, urogenital, cutaneous, ophthalmic, and other sites are all potential sites of EPTB. Although 15-20% of all tuberculosis cases are EPTB[3]. A new, less expensive test is needed for tuberculosis detection as there is currently no one test that can be used to identify the disease in underdeveloped nations such as India. Time, inconvenience, and a lack of sensitivity are just a few of the problems with traditional diagnostic methods. Because of this, tuberculosis (TB) tests and treatments are typically delayed [2]. There has been an upsurge in tuberculosis (TB)-related morbidity and death as a result of the absence of efficient and quick diagnostic technologies.

Although it is the most common and sometimes the only test available, smear microscopy only catches 45% of tuberculosis infections [4,5]. This is despite the fact that 95% of tuberculosis cases and deaths occur in impoverished countries. Despite its low PPV and moderate sensitivity of 80%, smear microscopy by ZN method remains the gold standard for tuberculosis diagnosis in situations with limited resources [6]. Drug susceptibility testing and the "gold standard" for final determination are both made possible by culture. The delay in treatment beginning is due to the 2-6 week duration, but [7].

The turnaround time and speed of Nucleic Acid Amplification Testing (NAAT) on direct samples are both higher than those of culture. In nations where tuberculosis is common, the high cost of polymerase chain reaction (PCR) testing prevents its widespread use [2]. With high sensitivity and specificity, IS6110 is the most often utilized target in PCR tests for diagnosing pulmonary and EPTB [8]. More affordable and faster diagnostic methods are required, particularly in India. One innovative approach is Truenat, a battery-operated RT-PCR system that uses a chip to detect MTB at the point of care. It allows for quick diagnosis with a little sample size, making it useful in areas with limited infrastructure. A second chip may be used as a follow-up test to determine if Truenat MTB 'detected' samples have rifampicin drug resistance. In comparison to GeneXpert, the Truenat MTB test is very sensitive and specific for the early detection of EPTB and pulmonary tuberculosis [17]. On the other hand, investigations validating it against culture techniques using extra-pulmonary material are few. Since tuberculosis (TB) is more common in developing states like Bihar, the PPV is likely to rise as well. Consequently, it is critical to assess Truenat MTB's accuracy in diagnosing tuberculosis (TB), particularly EPTB, which is significantly more common. In order to diagnose EPTB, this research compared the Truenat MTB test to microscopy and culture.

MATERIALS AND METHODS:

Over the course of fifteen months, researchers at IGIMS in Patna, Bihar, India, collected data for this cross-sectional study. The evaluation included 254 clinical samples taken from locations other than the lungs. **Inclusion Criteria:** The trial included all patients with presumed EPTB who were

at least one year old, had a prior history of anti-tubercular therapy (ATT), and whose treatment had failed during their most recent session of treatment. There are several kinds of extrapulmonary tuberculosis (EPTB), including those affecting the lymph nodes, pleura, abdomen, central nervous system, pericardium, spinal cord, bones and joints, genitourinary system, eyes, tuberculous otitis media, and skin.

Exclusion Criteria: This research did not include individuals who were on ATT or who had pulmonary TB.

Evidence of EPTB: Microbiologically confirmed cases of EPTB were defined as any clinical sample that showed the presence of MTB, either using the Truenat MTB test or positive culture/microscopy. If Truenat could not identify Mycobacterium tuberculosis (MTB), then any sample that tested positive with culture or microscopy was further tested for the presence of Non-Tuberculous Mycobacteria (NTM) with culture.

Clinical Specimen Processing: Blood, synovial fluid, pleural fluid, peritoneal fluid, urine, bone, central nervous system (CNS), liver, and pus samples from cold abscesses and deep locations were all taken from patients who were thought to have the disease. Centrifuged in a sterile tube at 5000 rpm (revolutions per minute) for five minutes were all the fluid specimens. After rinsing with sterile water, the tissue samples should be ground up using a micro pestle or mortar and pestle.

The standard methods of culture and microscopy: Following digestion, decontamination, and concentration, the samples were prepared according to established methods for MGIT culture and smear microscopy. For the first week, we checked the culture bottles every day; after that, we checked them once every week for eight weeks. Additionally, acid fast bacilli (AFB) were identified by ZN staining.

Trial of Truenat MTB Plus: Follow all directions on the product label while administering the Truenat MTB Plus test.

Before beginning the PCR run on a completely portable standalone thermal cycler, the user must add 5 ml of extracted DNA to a pre-loaded microchip [18] that contains chemicals stable at room temperature. The equipment in question is the Truelab UnoTM, a battery-operated mobile device. For real-time monitoring, the Truelab platform includes a portable device that houses the control electronics and optical detection system, a microprocessor with integrated temperature control components, and a personal digital assistant (PDA) that runs the software application. The Truenat MTB test utilizes a battery-operated sample preparation equipment called Trueprep-MAGTM. This device uses a nanoparticle-based methodology that is customized for each sample to extract nucleic acids via a user-friendly menu-driven process. The gadget allows nucleic acid separation without the need for extra equipment by integrating all operations (heating, fluid mixing, magnet control, step timing) using a programmable micro-controller and easy-to-follow screen instructions. The goal of developing this chip-based test was to make real-time polymerase chain reaction (PCR) as easy as possible, from "sample to result," so that even labs with limited resources may regularly run the tests and have their findings in under an hour.

Statistical analysis: When evaluating the Truenat MTB test, its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and observed agreement were compared to those of other tests. We used summary statistics with a 95% confidence interval to show the data.

RESULT: Total 254 samples of extra-pulmonary were taken. Out of the 254, 26 (10.24%) patients were MTB positive by Truenat in which 5 were RIF resistant, 12 RIF indeterminant and 9 RIF sensitive.

Department	Number of samples (%)
General Surgery	92 (36.22 %)
Pulmonary Medicine	74 (29.13%)
Gastroenterology	56 (22.04%)
Orthopaedics	9 (3.54%)
Paediatrics	7 (2.75%)
General Medicine	5 (1.96%)
Neurosurgery	5 (1.96%)
Obstetrics and Gynaecology	2 (0.78%)
Cardiology	2 (0.78%)
Dermatology	2 (0.78%)
Total	254

[Table/Figure-1]: Sample distribution across several departments.

Age group (years)	Number of Samples	Number of Truenat MTB positive samples	
0-10	9	1	
11-20	21	5	
21-30	69	10	
31-40	34	7	
41-50	40	-	
51-60	30	1	
61-70	32	-	
71-80	12	2	
81-90	5	-	
91-100	2	-	
Total	254	26	

[Table/Figure-2]: Distribution of samples by age group. tuberculosis bacteria, or MTB

for short.

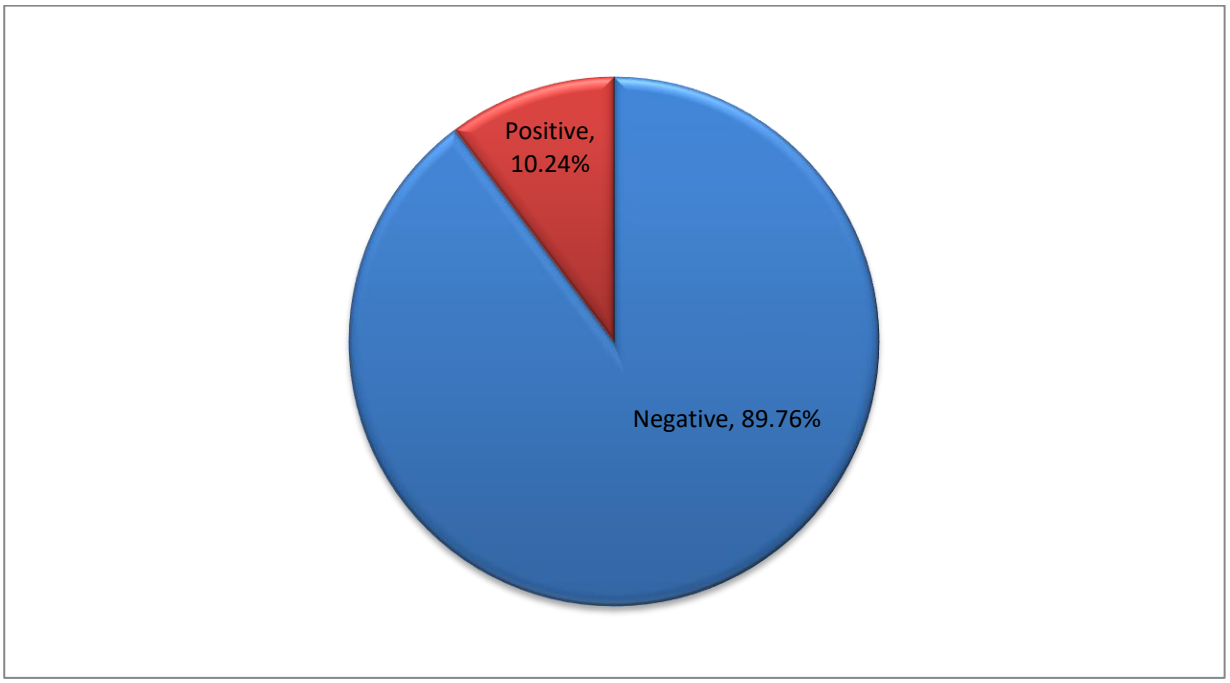
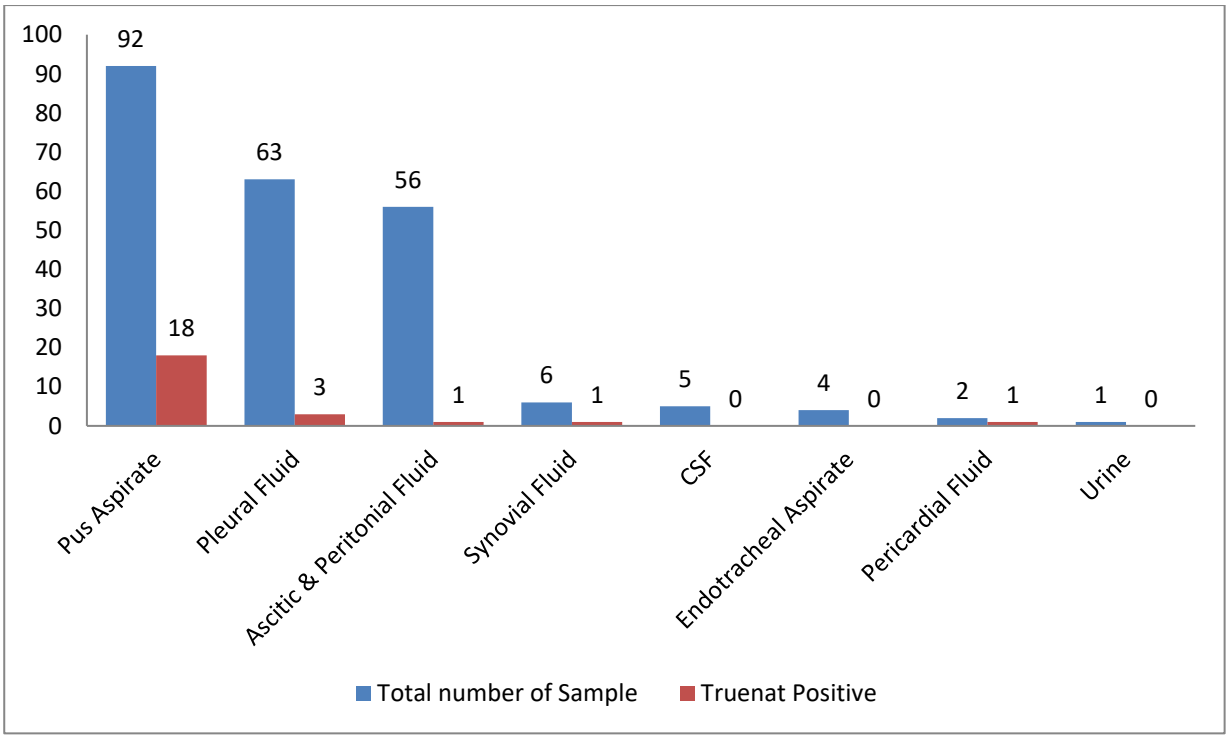
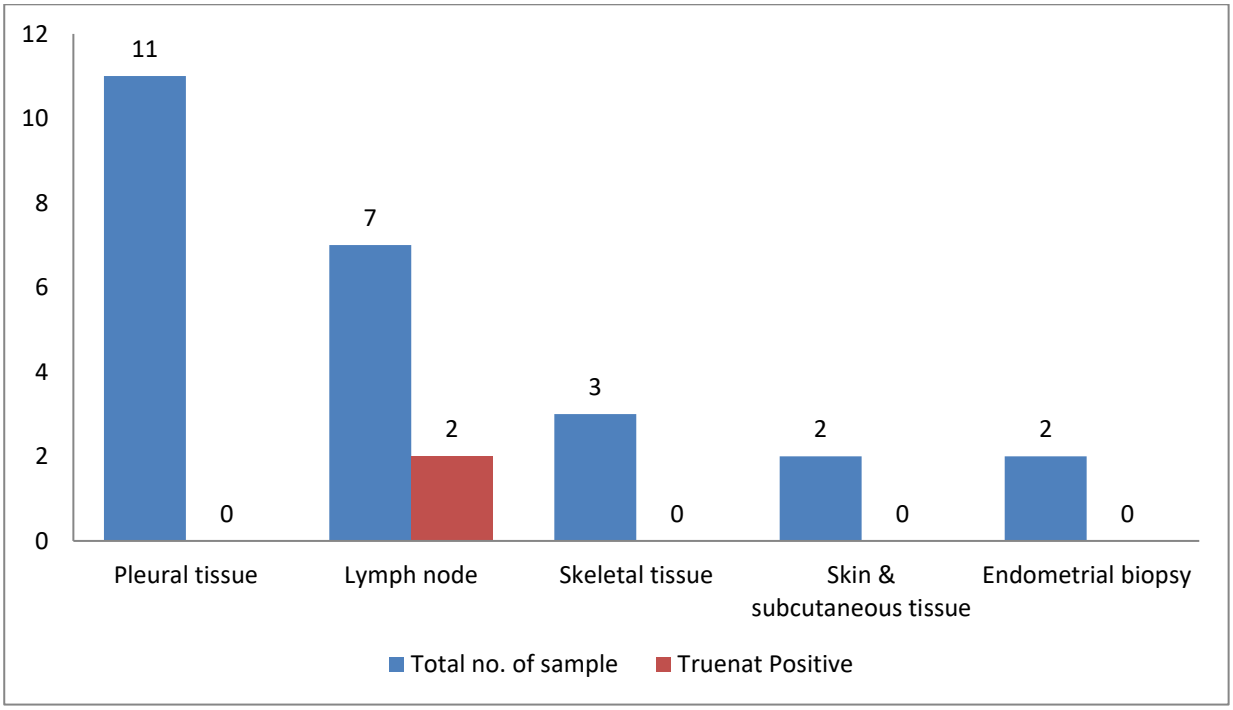


Figure-3: Mycobacterium tuberculosis Results.



[Table/Figure-4]: Distribution of fluid specimen types and their Truenat positivity. It stands for cerebrospinal fluid.



[Table/Figure-5]: The Truenat positivity distribution of different kinds of tissue and solid specimens.

TEST	**LQUID CULTURE**		
		P0SITIVE	**NEGATIVE**
TRUENAT	**P0SITIVE**	**24 (TRUE POSITIVE)**	**2 (FALSE POSITIVE)**
	NEGATIVE	**0 (FALSE NEGATIVE)**	**228 (TRUE NEGATIVE)**

[Table/Figure-6]: Comparison of Truenat with liquid culture.

Sensitivity-100%

Specificity-99.13%

TEST	**MICROSCOPY**		
		P0SITIVE	**NEGATIVE**
TRUENAT	**P0SITIVE**	**12 (TRUE POSITIVE)**	**0 (FALSE POSITIVE)**
	NEGATIVE	**14 (FALSE NEGATIVE)**	**228 (TRUE NEGATIVE)**

[Table/Figure-7]: Comparison of Truenat with microscopy.

Sensitivity-46.15%

Specificity-100%

DISCUSSION:

The results showed that Truenat was highly concordant when tested against microscopy and culture, demonstrating both sensitivity and specificity. emphasis contrast to previous research that relied on pulmonary samples, this one zeroed emphasis on extrapulmonary samples, the percentage of which is now on the rise [16,20]. The purpose of this research is to determine if Truenat is useful for the diagnosis of EPTB when used with extra-pulmonary specimens [24]. The microbiology lab of a tertiary care hospital in Patna, Bihar, India, received 254 samples of suspected tuberculosis patients from different clinical departments. Consistent with a previous research [25], a higher prevalence of EPTB in females was observed (14 out of 26 positives). Worldwide, men had a greater prevalence of tuberculosis, according to WHO statistics [26]. The age group of 21–30 had the highest number of confirmed cases at 9. Tuberculosis affects individuals of all ages, however adult males bear the brunt of the disease [25]. The most common kind of tuberculosis in this research was pus (18 cases, or 7.08%), followed by pleural (3 cases, or 1.18%), lymph node (2 cases, or 0.78%), and pericardial (1 case each, or 0.39%). Lymph node TB and pleural TB were the most prevalent types of EPTB in other investigations [25,27]. Ranjana H. and Sadhna S.'s investigation on the diagnosis of EPTB by Truelab MicroPCR found that endometrium was the most prevalent kind of sample [24]. An unmatched sensitivity of 100% and

specificity of 99.13% were shown by Truenat when tested with culture, the gold standard. The Truenat test was 46.15% sensitive and 100% specific when compared to microscopy. A related study by Nikam C et al. [17] indicated that when testing Truenat with sputum samples under microscopy, the sensitivity was 100% and the specificity was 43.98%. However, when testing Truenat with culture, the sensitivity was 94.70% and the specificity was 52.85%. According to a different research by Nikam C et al. [20], the Truenat MTB outperformed a Composite Reference Standard (CRS) in terms of speed and sensitivity when it came to TB detection. Although there was increased specificity as well, the current study's findings were consistent with the previous one. Unlike the current trial, which is likely the first of its sort in EPTB, the two studies that came before it used lung samples to determine how well Truenat MTB worked. Comparatively, the sensitivity of the Truenat MTB test is greater than that of smear microscopy and MTB cultures. This novel tool is important in a poor resource setting nation like India since it is indigenous, affordable, and convenient, according to the current research. In order to replace other molecular diagnostic procedures, this research lays the groundwork and opens the way for bigger investigations.

CONCLUSION(S)

For the diagnosis of EPTB in areas with limited resources, the Truenat MTB test provides an efficient and quick molecular diagnostic tool.

When it comes to the rapid and accurate diagnosis of EPTB, the Truenat MTB test is unrivaled. There was a lot of agreement between this research and previous ones on molecular diagnostic testing using the Truenat assay. Therefore, this test has the makings of a promising, quick, and accurate way to identify EPTB patients.

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