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Original Research Article

# Evaluation of Truenat MTB Test in Comparison with Ziehl-Neelsen staining and MGIT automated Liquid Culture for Diagnosis of Suspected Pulmonary and Extrapulmonary Tuberculosis in Paediatric Patients in a Tertiary Care Hospital

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**Abstract**

**Introduction:** The diagnosis of Paediatric tuberculosis is a big challenge, as the sample collection is difficult and number of *Mycobacterium tuberculosis* (MTB) bacilli in the samples often very low. TrueNat MTB plus assay is a newer method, which is battery operated, point-of-care and chip-based Real Time Polymerase Chain Reaction (RT-PCR) micro device.

**Aim:** To evaluate TrueNat as a screening test in the diagnosis of Pulmonary and extrapulmonary TB in comparison with ZN microscopy and MGIT culture.

**Materials and Methods:** A prospective cross-sectional study was carried out over 3 months of period in which 100 samples from suspected cases of paediatric tuberculosis were subjected to Ziehl-Neelsen (ZN) staining for smear microscopy, culture on MGIT automated culture media and PCR for MTB by Truenat. Comparisons were made between the tests and the data was presented using summary statistics with 95% Confidence Interval (CI).

**Results:** Out of 100 suspected cases, 24samples were detected positive by MGIT culture. Truenatdetected MTC in 22cases.7 cases detected positive by all the three tests performed including ZN stain, MGIT and PCR. MGIT culture positive was considered gold standard of diagnosis of tuberculosis. All samples positive through by ZN staining (7) were positive by MGIT automated culture system andTruenat. Sensitivity and specificity of Truenatwas 70.83% and 93.42 % respectively. ZN staining showed sensitivity of 29.17% and specificity of 100%. Conclusion: Our study shows promising sensitivity and specificity for TrueNat MTB plus assay in paediatric samples.

**Keywords:** Tuberculosis, Truenat, MGIT, Peadiatrics, ZN staining, Diagnosis.



## Introduction

Tuberculosis remains major global health burden till date. Due to recent COVID 19 pandemic, diagnosis and treatment of tuberculosiswas impacted due to lack of access of proper diagnostic modalities in many developing countries including India.[1] Undiagnosed cases of tuberculosis is increased to 4 million people in 2020 according to World Health organisation. [2] This leads us to find a rapid point of care modality which can be easily accessible and cost less than available diagnostic procedures. [3] Tuberculosis in young children poses great threat as it can lead to mortality very easily. [4] Recent report from WHO showed that under 15 children constitutes 14% of total mortality among the HIV negative TB deaths, [1] As major share of mortality among the children is due to not receiving proper treatment, one can think that it originated from diagnostic difficulties of paediatric tuberculosis. [5]

Difficulties of diagnosis of TB in paediatric age group starts with sample collection as children are often unable to produce proper non-invasive sample like an adult. It is also observed that Tuberculosis in childhood is paucibacillary in most of the cases which is a major obstruction for the diagnostic cause. [6] As for this hospitalisation and invasive procedure have become necessity for many cases and fewer cases of tuberculosis ended

up properly diagnosed. This leads to great burden of mistreatment and lack of scientific advancement in this field. [7] Smear microscopy by ZN staining is one of the major resources used for tuberculosis till date, although this method yields poor sensitivity for diagnosis. [8] Mycobacterial culture in other hand considered as gold standard for the diagnosis although it takesup to 6 weeks to give proper result. [9] The TrueNat MTB Plus assay (Molbio Diagnostics, India) is a recent molecular development of tuberculosis testing. Itbased on the portable, battery-operated chip bases system which can be easily operated without illustrious setting. [10,11]. WHO recommended TrueNat as diagnostic method along with culture and microscopy in 2020 along with use the resourcefulness of the detecting Rifampicin susceptibility in the sample. [12]

In this study we tried to assess the sensitivity and specificity of TrueNatMTB Plus assay and ZN staining in comparison to automated MGIT liquid culture system in suspected pulmonary and extrapulmonary tuberculosis cases of paediatric age group. (Under 15 years) in a tertiary care hospital in India.

## Methodology

This was a prospective cross-sectional study. It was done in a tertiary care centre in New Delhi, India during the period from November 2022to January 2023. Total 100 pulmonary and extrapulmonary clinical samples were obtained from the paediatric age group patient (<15 years ) during this period. Patients who were on ATT were excluded from the study.

**Sample Processing:** Different specimens like Sputum, Broncho alveolar lavage (BAL), Gastric aspirate (GA), Lymph node biopsies, tissue biopsies, pleural fluid, synovial fluid, CSF, pus were obtained from suspected cases after getting informed consent from the patient's guardians. The tissue specimens were homogenised by using mortar and pestle.Sputum was processed by using the N-acetyl-L-cysteine–NaOH (NALC-NaOH). All samples were subjected ZN staining, MGIT 960 automated culture system and TrueNat MTB Plus assay.

**Microscopy:** Ziehl-Neelsen method is used for acid fast staining as per current guideline available through National tuberculosis Elimination programme of India. (13)

**Culture:** The MGIT (Mycobacteria Growth Indicator Tube) (Becton Dickinson, New Jersy, USA) consisted of liquid broth medium. The MGIT contained 7.0 ml of modified Middlebrook 7H9 broth base. Using sterile pipette 0.5 ml of decontaminated sample was added in appropriately labeled MGIT tubes. All the inoculated tubes were placed in BACTEC MGIT 960 instrument at 37ºC temperature. Indicator light in the Instrument signaled when a tube gave positive growth. After 6 weeks instrument will flag negative if there is no growth. We followed manufactures instruction for detailed procedure.

**TruenatMTBPlus test:** Manufacturer's instructions were followed during conducting TrueNat MTB Plus test.

The extraction of (DNA) from the samples was completed using Trueprep AUTO Universal Cartridge Based Sample Prep kit and device. The pre-treated sample was transferred to the sample chamber of the cartridge and was placed in the device. An elution collection tube was used for the collecting elute from each extraction.

6µL of the extracted material was transferred into TrueNat Microchip which contained lipolyzed master mix for PCR. Real time PCR was conducted and results were displayed as 'Detected' in positive cases and 'Not Detected' in case of negative results.

**Statistical Analysis:** Comparison of the TrueNatMTB and ZN staining were done with MGIT 960 automated culture system. Statistical analysis was done using statistical software Epi Info (version 7.2.5). We used 95% confidence interval to present the data. Sensitivity, specificity, positive predictive value and negative predictive value were calculated for both TrueNat and ZN staining.

## Results

Total 100 sample, both pulmonary and extrapulmonary were obtained from suspected cases of both pulmonary and extrapulmonary tuberculosis in paediatric ward and OPDs. Most of the cases in this study is female (56%), where males contributed 44% of cases.

Among different samples, sputum was predominant (49%), followed by gastric aspirate (38%). Distribution of sample types and their positivity is shown in Table 1 and Figure 1.

**Table 1:**

| Type of Sample | No. of Samples | Positive by ZN Staining | Positive by MGIT | Positive by TRUNAT |
|---|---|---|---|---|
| BAL | 4 | 0 | 2 | 2 |
| CSF | 3 | 0 | 1 | 2 |
| Gastric Aspirate | 38 | 1 | 6 | 4 |
| Lymph Node | 2 | 0 | 0 | 0 |

| | | | | |
|---|---|---|---|---|
| Sputum | 49 | 6 | 13 | 12 |
| Tissue | 1 | 0 | 0 | 0 |
| Pus | 2 | 0 | 2 | 2 |
| Pleural Fluid | 1 | 0 | 0 | 0 |
| Total | 100 | 7 | 24 | 22 |

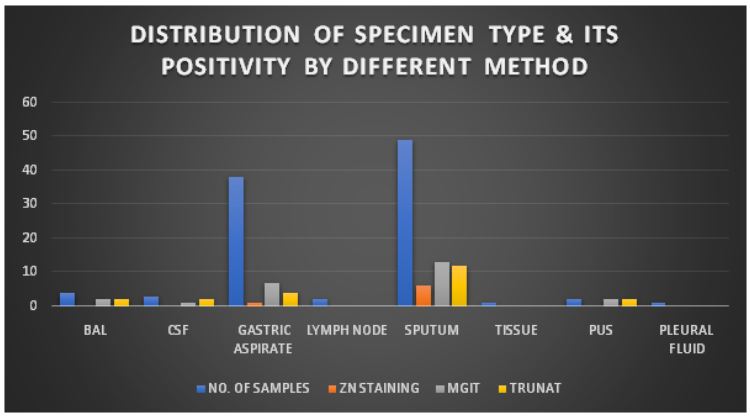


**Figure 1:**

In this study, 22% cases found positive through TrueNat and24% cases was detected positive by MGIT There was a significant difference in the distribution of test results between TrueNatand MGIT. (P value < 0.05) (Table 2)

**Table 2:**

| | **TRUENAT** | | **Total** |
|---|---|---|---|
| MGIT | Positive | Negative | |
| Positive | 17 | 7 | 24 |
| Negative | 5 | 71 | 76 |
| Total | 22 | 78 | 100 |

**Table 3:**

| **Statistic** | **Value** |
|---|---|
| Sensitivity | 70.83% |
| Specificity | 93.42% |
| Positive Predictive Value | 77.27% |
| Negative Predictive Value | 91.03% |

When Zn staining was compared with gold standard, there is a significant difference of the result observed. (P value < 0.05) (Table 3)

**Table 4:**

| | **ZN STAINING** | | **Total** |
|---|---|---|---|
| MGIT | Positive | Negative | |
| Positive | 7 | 17 | 24 |
| Negative | 0 | 76 | 76 |
| Total | 7 | 93 | 100 |

**Table 5:**

| **Statistic** | **Value** |
|---|---|
| Sensitivity | 29.17% |
| Specificity | 100.0% |
| Positive Predictive Value | 100.0% |
| Negative Predictive Value | 81.72% |

## Discussion

In our investigation, we used 100 suspected instances of paediatric tuberculosis, and when the TrueNat MTB assay was compared to the gold standard culture method, it showed a sensitivity of 70.83%. ZN staining achieved a sensitivity of 29.17% in the same setting. This is notified that there is a scarcity of literature regarding this kind of study among paediatric population.

Compared with a study conducted among children at AIIMS, New Delhi, which concluded with 57.12% sensitivity for TrueNat, our study shows significantly higher sensitivity (70.83%). Although it was noticed specificity were similar in both cases. We should point out that the other study only collected pulmonary samples. [14]

Comparing both parameter in case of extrapulmonary tuberculosis, a study by Jose RE et al figured-out sensitivity and specificity of TrueNat MTB assay is 100% and 95.1%. It was observed that ZN microscopy also produced a high sensitivity and specificity (100% and 96.9%).Both resultsshowed higher sensitivity than our study. [15]

Another multicentric investigation carried out in Cameroon revealed that the sensitivity of the TrueNat test was 91% as opposed to the 70.8% sensitivity of the TrueNat in our study. Unlike their study, which only included sputum samples from adult patients, ours also included extrapulmonary samples and paediatric patients. [16]

Additionally, we discover that the TrueNat test has a 93.42% specificity, which is better than that of Nikam C. et al.'s investigation on lung samples, which found a specificity of 52.85% for TrueNat. [17]

TrueNat MTB plus assay uses*nrdZ* gene and the multicopy IS6110 insertion element for detection of the tuberculosis. As IS6110 is a specific sequence for the *Mycobacterium tuberculosis complex*, trueNAT assay provide an extract edge of sensitivity during testing. According to manufacturer's guideline, TrueNat MTB plus assay can detect if there is up to 30 CFU/ml. [18.19]

TrueNat assay also take a shorter time (2 -3 hours) compared to Culture method which is considered as gold standard. As presented TrueNat assay is good and readily available as point of care diagnostic test for diagnosis tuberculosis in children even in primary and secondary health care setup.

This study is limited to a smaller population and conducted over a short period of time. Another shortcoming is result of rifampicin sensitivity is not included in this study.

## Conclusion

Simple, rapid and sensitive approach is needed to diagnose tuberculosis in children as it's often neglected and underdiagnosed in this country. Our study shows promising sensitivity and specificity for TrueNat MTB plus assay in paediatric samples. We hope this might provide a new insight to elimination of tuberculosis in upcoming days.

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