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# Detection of *Mycobacterium tuberculosis* by EasyNAT™ Diagnostic Kit in Sputum Samples from Tanzania

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## INTRODUCTION

- Early and accurate diagnosis of tuberculosis (TB) and treatment are the mainstay of TB control. Smear microscopy, a sole diagnostic tool in resource limited settings, has low sensitivity particularly in settings with HIV burden.
- Nucleic acid amplification tests (NAATs) for TB are high-performing tests for detecting TB RNA and DNA in clinical samples.
- The EasyNAT™ tuberculosis isothermal nucleic acid amplification diagnostic kit by Ustar Biotechnologies Co., Ltd. uses isothermal cross-priming amplification technology for the qualitative detection of *Mycobacterium tuberculosis* (*M. tuberculosis*).

## OBJECTIVE

- To evaluate the diagnostic performance of EasyNAT in detecting *M. tuberculosis* from sputum smears of presumptive pulmonary TB patients in Bagamoyo, Tanzania

## METHODS

- From a TB cohort study of presumptive TB patients, one ml of frozen fresh untreated morning or spot sputum samples was used to evaluate EasyNAT against Ziehl Nielsen (ZN) smear microscopy, BACTEC Mycobacterium Growth Indicator Tube (MGIT) 960 and Löwenstein Jensen (LJ) culture.
- Molecular genotyping (Genotype MTBC, CM or AS; Hain Lifescience, Nehren) and MPT64 antigen confirmed *M. tuberculosis* or non-tuberculous mycobacteria.

## RESULTS

- We analyzed sputum samples of 143 presumptive TB patients (Figure 1).
- The mean age was 40.5 years (standard deviation=15.3); 78 (54.6%) were men.
- The HIV prevalence was 46.2% (95% Confidence Interval [95% CI]: 37.8%-54.7%). The HIV prevalence in culture-confirmed TB patients and controls was 47.9% and 42.7%, respectively (P=0.568, chi-square test).
- The sensitivity of EasyNAT against culture as a reference standard was 66.7% (95% CI: 51.6%-79.6%). All Controls (no symptoms at 5 months of follow-up and an alternative diagnosis established) were EasyNAT-negative (specificity 100%, 95% CI: 95.2%-100%) (Table 1).
- One of the 10 smear-negative and culture-positive TB patients was EasyNAT-positive (sensitivity 10%, 95% CI 0.3%-44.5%).
- No *M. tuberculosis* was detected by the EasyNAT assay in 10 patients with clinically diagnosed TB and in 10 patients who had the following *Mycobacterium* species and strains: *M. fortuitum* strain 1, *M. fortuitum* strain 2/*M. mageritense*, *M. malmoense*/*M. haemophilum*/*M.pasture*, *M. celatum* I/III, *M. simiae*, *M. celatum*, *M. intracellulare, M. asiaticum*, *M. scrofulaceum,* or *M. smegmatis.*

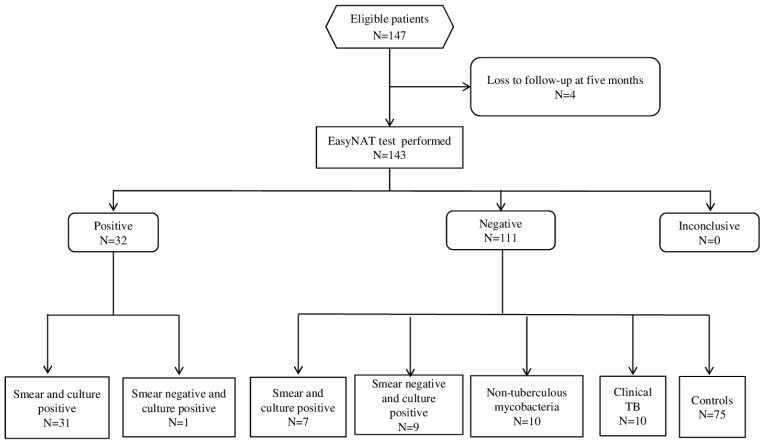


**Figure 1:** Patient flow and EasyNAT test results by patient classification

**Table 1:** Performance of EasyNAT with smear microscopy, MGIT, LJ and MGIT & LJ combined vs. controls as reference standards

| | Main Analysis | Sub-Analysis | | |
|---|---|---|---|---|
| **Reference standard** | Culture MGIT &LJ vs. controls | Smear microscopy vs. controls | Culture MGIT alone vs. controls | Culture LJ alone vs. controls |
| **Per patient analysis** | Estimate (95%CI) | Estimate (95%CI) | Estimate (95%CI) | Estimate (95%CI) |
| **Sensitivity** | 66.7 (51.6-79.6) | 81.6 (65.7-92.3) | 66.7 (51.6-79.6) | 69.2 (52.4-83.0) |
| **Specificity** | 100 (95.2-100.0%) | 100 (95.2-100.0%) | 100 (95.2-100.0%) | 100 (95.2-100.0%) |
| **Positive Predictive Value** | 100.0 (89.1-100.0) | 100 (88.8-100.0%) | 100.0 (89.1-100.0) | 100.0 (87.2-100.0) |
| **Negative Predictive Value** | 82.4 (73.0-89.6) | 91.50 (83.2-96.5) | 82.40 (73.0-89.6) | 86.2 (77.1-92.7) |
| **Positive Likelihood Ratio*** | | - | - | - |
| **Negative Likelihood Ratio** | 0.3 (0.2-0.5) | 0.19 (0.1- 0.4) | 0.3 (0.2-0.5) | 0.3 (0.2-0.5) |

* The positive likelihood ratio could not be computed since is given by sensitivity/(1-specificity). In all cases, the specificity was 1 (or 100%).

## CONCLUSION

- The EasyNAT assay detected *M. tuberculosis* with an excellent specificity and positive predictive value. The sensitivity was acceptable in the smear-positive patients.
- However, the low detection rate for the smear-negative, culture-positive sputum samples might be a limitation for wider clinical use and requires further evaluation in larger study populations from different regions that are endemic for TB.

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