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DIAGNOSIS OF PULMONARY TUBERCULOSIS BY MICROSCOPIC OBSERVATION DRUG SUSCEPTIBILITY ASSAY IN HIV-POSITIVE PATIENTS

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Abstract

Microscopic observation drug susceptibility assay (MODS) is a novel and promising test for the early diagnosis of tuberculosis (TB). We evaluated the MODS assay for the early diagnosis of TB in HIV positive patients presenting to Pham Ngoc Thach Hospital for Tuberculosis and Lung Diseases in southern Vietnam. 738 consecutive sputum samples collected from 307 HIV-positive individuals suspected of TB were tested by smear, MODS and MGIT. The diagnostic sensitivity and specificity of MODS compared to microbiological gold standard (either smear or MGIT) was 87% and 93%, respectively. The sensitivity of smear, MODS and MGIT were 57%, 71% and 75%, respectively against clinical gold standard (MODS vs smear: P<0.001, MODS vs MGIT: P=0.03). Clinical gold standard was defined as patients who had clinical examination and treatment consistent with tuberculosis, with or without microbiological confirmation. For diagnosis of smear negative patients, the sensitivity of MODS and MGIT were 38% and 45%, respectively (P=0.08). The median time to detection of MODS and MGIT were 8 days and 11 days, respectively, and 11 and 17 days, respectively, for smear negative samples. Original bacterial/fungal contamination rate of MODS was 1.1% while it was 2.6% for MGIT. The cross-contamination rate of MODS was 4.7%. In conclusion, MODS is a sensitive, specific and rapid test which is appropriate for detection of HIV-associated TB; the cost and ease of use make it particularly useful in resource limited settings.

Introduction

It is estimated by the World Health Organisation (WHO) that there were 9.4 million new cases of tuberculosis (TB) in 2008 (3). Of these, 1.4 million (15%) were in HIV-positive patients and 23% of all HIV deaths are estimated to be attributable to TB (2).

Viet Nam is a high TB burden country with steeply rising rates of HIV-TB co-infection (28); 8.1% of newly diagnosed TB patients are now HIV infected (3). These cases are the most urgently in need of diagnosis because they have the highest morbidity and mortality yet the diagnosis of TB among HIV infected individuals is difficult. Screening algorithms based on clinical symptoms alone show high sensitivity but low specificity (4, 12). Microscopy smear while simple, specific and widely available in high burden settings, has particularly lowsensitivity in HIV patients and cannot be used to rule out a diagnosis of TB (20, 27). Microbiological confirmation remains desirable and allows investigation of drug susceptibility profiles. Commercial rapid liquid culture techniques have been endorsed by WHO (6), show higher sensitivity and are more rapid than traditional solid media-based techniques such as Lowenstein-Jensen culture. However, their high cost and biosafety infrastructure requirements limit their applicability in many high burden settings. Rapid molecular line-probe assays, also endorsed for use in low-resource settings by WHO (7), allow simultaneous identification of *M. tuberculosis* and resistance to rifampicin or isoniazid but are currently only recommended for smear-positive samples and positive cultures. In addition, they are expensive and require molecular expertise which is often not available in low-resource settings.

Recent evaluations of a novel diagnostic test for TB, Microscopic Observation Drug Susceptibility assay (MODS) have shown it to be economical and rapid with a turn around time

of 7 days, making it ideal for use in high burden, low-resource settings (9, 10, 21). MODS has been shown effective in identification of TB in HIV patients (9, 25). The increasing number of HIV-positive pulmonary TB suspects presenting to Pham Ngoc Thach hospital, a referral TB hospital in the south of Viet Nam, has led to an urgent need for a rapid and sensitive test to detect TB for this population. Here, we evaluated the MODS assay as a promising method for TB detection. We assessed the sensitivity, specificity, negative predictive value, positive predictive value, contamination rate and turn around time of MODS against clinical gold standard and microbiological gold standard.

Methods

Enrollment: All HIV-positive individuals suspected of tuberculosis, who were newly presenting to the HIV/TB ward at Pham Ngoc Thach Hospital from May to November 2008 were enrolled into the study unless they had received >8 days TB therapy. Data on socioeconomic and demographic features, TB history, TB contact history, HIV status and presenting clinical features were prospectively collected on a standard case report form. Samples were collected as per routine care as deemed appropriate by the treating physician (usually 3 in accordance with WHO recommendations). No additional samples were collected as part of this study and only sputum samples were evaluated. The definition of TB was based on microbiological confirmation by either smear or MGIT, intention to treat, treatment management and outcome**.** Tuberculosis was defined as "confirmed TB' if the patient had clinical symptoms consistent with TB (1) and either smear or MGIT was positive in any sample, including samples which were collected before the enrollment started. These samples were not included in the sensitivity comparison but patients with prior samples positive in this illness episode by either smear or MGIT were classified in the

"confirmed TB" group. A positive MODS culture was not considered as part of the definition of 'confirmed TB' because this was the test under evaluation.

The patient was defined as "probable TB" on 'intention to treat' if the patient had clinical symptoms consistent with TB (1) but had no microbiological confirmation, received no alternative diagnosis and initiated TB treatment and transferred to a District Tuberculosis Unit for treatment and follow-up. Patients who satisfied the first two characteristics of "probable TB" but self-discharged prior to treatment were also classified in this group if the clinician intended to treat for TB. It was impossible to either rule-out or confirm TB in this group due to the lack of microbiological confirmation.

Patients were defined as "TB unlikely" if they recovered without TB treatment, had TB treatment but deteriorated or received an alternative diagnosis and treatment. It was impossible to 'rule-out' TB in these patients completely because clinical deterioration on therapy may have been due to undetected drug-resistant TB.

Ethics: The protocol was approved by the Institutional Review Board (IRB) at Pham Ngoc Thach Hospital and the Health Services of Ho Chi Minh City. Individual informed consent was not sought because the study was conducted on routine samples only and it did not involve any intervention, additional samples or change in patient management. A patient consent waiver was approved by the IRB of Pham Ngoc Thach Hospital.

Sample collection: All sputum samples were collected and transferred to the microbiology department on the same day (or the following day if they were collected after 4pm). The samples were then submitted for smear, MGIT and MODS culture. The number of specimens per patient was decided by the treating physician.

Sample processing: Sputum samples were homogenised and decontaminated by Sputaprep (NaOH-NALC 2%) manufactured by Nam Khoa Company, Viet Nam prior to testing. The kit contains Mucoprep (NaOH 0.5M and Na_3Citrate 0.05, NALC (N-Acetyl-L-Cysteine) and Phosphate buffer (PO_4 10X - 0.67M). Phosphate buffer 1X, homogenization buffer and decontamination buffer (HDB) were then prepared from the kit for sample processing. In brief, 3 – 5ml sample was added to 3 – 5ml HDB contained in a 50ml falcon tube. The tube was shaken lightly by automated shaker and left at room temperature for 20 minutes. After that, 35 – 39ml phosphate buffer 1X was added into the mixture. The mixture was shaken by hand and then centrifuged at 3000g, 4^0C for 30 minutes. The supernatant was then discarded and 0.5ml pellet at the bottom was re-suspended with 2ml distilled water. The deposit was then aliquoted into 3 parts for smear, MGIT culture and MODS.

Homogenous smear: Two drops of pellet from each sample were put onto a slide for homogenous smear preparation. The smears were then stained by ZN method according to WHO standard protocol (5).

MGIT culture: Processed samples were subjected to MGIT culture following the protocol of Becton Dickinson (BACTEC™ MGIT™ 960 Mycobacerial Detection System). In brief, 0.1ml PANTA, 0.5ml OADC and 0.5ml of each processed sample were added into a MGIT medium tube. The mixture was inversely mixed by hand and then inoculated and incubated at 37^0C in the MGIT machine. Positive results were reported automatically by the MGIT system. A smear from MGIT positive culture was made to confirm acid-fast bacilli.

MODS technique: The MODS culture was conducted in a biosafety cabinet class I which was placed in a separate room from the sample processing room, smear preparation room and MGIT

124 culture room. The MODS method was performed as described in Park *et al.* (22) using the minor
125 modifications described by Caws *et al*. (13). Briefly, MODS media was prepared with 5.9 g
126 Middlebrook 7H9 broth (Difco, Sparks, MD), 3.1 ml glycerol and 1.25 g bacto casitone (Difco,
127 USA) in 880 mls sterile distilled water. The media was autoclaved and stored in 22 ml aliquots at
128 4^0C. Each new batch was tested for sterility by incubating one aliquot at 37^0C for 1 week. Before
129 use, OADC and PANTA (Becton Dickinson, USA) were added into each tube to final
130 concentrations of 5.5% and 0.22% to make working MODS media. One 48-well MODS plate
131 (Becton Dickinson, USA) was set up each day. Seven hundred and fifty µls of working MODS
132 media was aliquoted to each well and 250 µl processed sample was added. One positive control
133 (H37Rv) and one negative control well (sterile distilled water) were inoculated to each plate.
134 Samples were inoculated into alternate wells to reduce cross-contamination. Empty wells
135 contained MODS media. To prevent cross-contamination from evaporation and ensure safety
136 plate seals (optical flims, Biorad) were used. The plate was further sealed with sellotape and
137 placed inside a Tupperware box, then incubated at 37^0C , and the plate examined every alternate
138 day after five days of inoculation for evidence of growth. Contamination was recorded if there
139 was any growth or turbidity in any negative control well.

140 **Subculture on LJ:** All cultures positive by MODS or MGIT were subcultured on LJ media
141 (Becton Dickinson) in duplicate and incubated at 37^0C for several weeks. These isolates were
142 then subjected to standard biochemical identification tests, DNA extraction (17) and archiving.

143 **Spoligotyping:** Spoligotyping was performed according to the standard international
144 Spoligotyping protocol (18) for all cultures positive by MODS (n=396). If MODS was
145 contaminated during subculture from MODS to LJ for DNA extraction (n=20) or MODS was
146 negative but MGIT positive (n=55), cultures positive by MGIT were used for spoligotyping.

147 Multiple isolates from the same patient were compared to identify discrepant spoligotypes. If a
148 single positive culture was obtained from a patient, samples processed on the same plate were
149 compared to identify probable cross-contamination. Cross-contamination of MGIT was not
150 addressed in this study due to resource limitations.

151 **Statistical methods:** Accuracy measures of the 3 tests were calculated for two different
152 definitions of the 'gold standard' reference test: (1) microbiological confirmation (confirmed
153 group) or (2) 'clinical diagnosis' (clinical gold standard including the probable and the
154 confirmed group). In addition, we analyzed data on a 'per patient' or a 'per sample' basis.

155 For the 'per patient' analysis, the data was aggregated to provide one result per patient, i.e. the
156 'per patient' test was regarded as positive, if at least one sample yielded a positive test result.
157 Reported confidence intervals for accuracy measures (sensitivities, specificities, positive and
158 negative predictive values) were calculated according to the method of Pearson and Clopper.
159 Comparisons of accuracies between tests were done using McNemar's test.

160 In the 'per sample' analysis we used a binary marginal generalized linear regression (GLM)
161 models with an identity link function for all analyses. These models are very flexible, allow for
162 the inclusion of covariates and account for the fact that results of multiple samples from the same
163 patient or test results of different tests on the same sample may be dependent (23). Specifically,
164 we used marginal regression model to calculate confidence intervals for accuracy measures, to
165 compare the sensitivities of smear, MGIT, and MODS and to assess the impact of the duration of
166 TB treatment on the sensitivity of MODS.

For the 'per sample' analysis, we also calculated time-dependent sensitivity curves for MGIT and MODS. A test result was considered as positive by time *t* if the respective test was positive overall and reached the positive value at most *t* days after sample collection. Time-dependent sensitivity curves were estimated with the Kaplan-Meier method and samples without a positive test result were formally regarded as censored on day "infinity". Time-dependent sensitivities of MGIT and MODS by days 7 and 14, respectively, were compared using a marginal regression model as described above. In addition, the time to positive MGIT and MODS, respectively, was compared in samples were both tests reached positivity with the Cox proportional hazards regression model. Robust sandwich estimators of the standards errors were used to adjust for possible dependence of multiple samples from the same patient or test results of different tests on the same sample.

Comparison of demographic and clinical features of patients between TB diagnoses (definite, probable or unlikely) was done with Fisher's exact test for categorical data and the Kruskal-Wallis test for continuous data.

All reported confidence interval are two-sided 95% confidence intervals and p-values ≤ 0.05 were regarded as statistically significant. All analyses and graphs were generated with Stata version 9 (Statacorp, Texas, USA)

RESULTS

341 HIV positive individuals were screened for pulmonary tuberculosis (***Figure 1***). Of these, 8.2% (n=28/341) patients were excluded because they subsequently tested HIV negative (3 cases), no samples were collected (24 cases) or they had already received TB treatment for more than eight days (1 case). Thus, 313 patients were eligible for the analysis. However, six

189 additional patients were excluded after clinical and laboratory analysis because insufficient
190 information was collected prior to self-discharge of the patient (4 cases) and inappropriate
191 sample (gastric fluid) was collected (2 cases). Thus, data from 307 patients were analyzed and
192 reported in this study.

193 A total of 738 sputum samples were collected from these 307 patients. Two hundred and twenty-
194 two (72%, n=222/307) patients had microbiological confirmation by a method other than
195 MODS. This group also included 6 patients with microbiological confirmation by smear or
196 MGIT based on samples collected prior to study enrolment. 61 patients (20%, n=61/307) were
197 classified as 'probable TB' and 24 patients (8%, n=24/307) as 'TB unlikely'.

198 **Demographics and clinical features:** Over 90% (n=301/307) of the study population was male
199 with a median age of 29. Almost 60% (n=182/307) patients had BCG vaccination determined by
200 BCG scar. Only 13% (n=42/307) were on antiretroviral (ARV) therapy. Twenty percent
201 (n=61/307) of patients had previously been diagnosed with TB once in their medical history.
202 ***Table 1*** shows demographic characteristics of the study population and comparisons of the three
203 groups. **Table 2** shows clinical features of the 307 HIV-associated TB suspects. Cough, fever
204 and weight loss were the most frequent symptoms with the majority of patients having a history
205 of illness between 30-59 days Lymphadenopathy was present in 43% of patients.

206 **Accuracy of MODS**

207 **Accuracy against microbiological confirmation as the gold standard:** Microbiological gold
208 standard was defined as patients whose samples were positive by either smear or MGIT. MODS

detected 87.4% of these cases with a specificity of 93%. The accuracy of MODS against microbiological gold standard, by patient and sample analysis, is detailed in **table 3.**

Accuracy against clinical gold standard: Clinical gold standard was defined as patients satisfying the definition of "microbiological confirmation" group (n=222 patients) or "Probable TB" group (n=61 patients). In total, 283 patients and 684 samples were classified as TB using the clinical gold standard. Table 4 describes the sensitivity and negative predictive value of MODS, Smear and MGIT against clinical gold standard by patient and by sample analysis. MODS was significantly more sensitive than Smear (71% vs 57%, p<0.001 by patient analysis and 64% vs 54%, p<0.001 by sample analysis) but less sensitive than MGIT (75%, p=0.03 by patient analysis and 70%, p<0.001 by sample analysis). The specificity and positive predictive value of all methods were 100%.

MODS in diagnosis of smear-negative HIV-associated TB : One hundred and twenty-two patients with 315 samples were diagnosed with confirmed or probable TB but all their smear samples were negative. Of which, 15/122 (12%) patients were positive by MGIT only, 40/122 (33%) patients were positive by both MODS and MGIT and 6/122 (5%) patients were positive by MODS only. MODS detected 72.8% (n=40/55) of culture positive-smear negative TB cases. Comparisons of the sensitivity and negative predictive value of MODS and MGIT are detailed in table 4. The sensitivity of MODS tended to be lower than MGIT in the 'by patient' analysis (38% vs 45%, p=0.078). Conversely, MGIT was significantly more sensitive than MODS (36% vs. 29%, p=0.003) in the "by sample" analysis. The specificity and positive predictive value of these tests in smear negative patients/samples were 100%.

Of 122 smear negative TB cases, 30 cases did not have TB therapy at Pham Ngoc Thach Hospital because of death, self-discharge or referral to District Tuberculosis Units to start TB treatment and follow-up. Ten out of these 30 cases (33%) did not receive TB treatment because the patient was discharged following a negative smear before the culture results were available.

TB treatment-dependent sensitivity: 684 samples from 283 patients with a clinical TB diagnosis were analyzed. 14% (97/684) samples were from patients not on TB. 540/587 (92%) samples were collected from patients on TB treatment ≤ 3 days and 47/587 (8%) samples were from patients on TB treatment >4 days. The sensitivity of MODS, Smear and MGIT against clinical gold standard in patients receiving TB treatment ≤ 3 days or ≥ 4 days was compared. The sensitivity of MODS and smear were significantly decreased among samples collected after 4 days of TB treatment compared to earlier samples (53% vs 70%, p=0.035 for MODS and 45% vs 61%, p=0.034 for Smear); The sensitivity of MGIT also tended to be lower for longer TB treatment duration but the result did not achieve statistical significance (74% vs 60%, P=0.053).

Time to positive: Time to positive was defined as the number of days from sample processing (day1) to result available. The results of Smear were available on day 2 (routine procedure at Pham Ngoc Thach Hospital). In samples positive by either MODS (n=437/684) or MGIT (n=473/684), the median time to detection of MODS and MGIT were 8 days (IQR: 6 – 10days) and 11 days (IQR: 8 – 10 days), respectively. Among smear negative samples, the median time to detection of MODS and MGIT were 11 days (IQR: 9 – 16 days) and 17 days (IQR: 13 – 21 days).

Time-dependent sensitivity: Time-dependent sensitivity of MODS and MGIT are shown in ***Figure 2***. In samples positive by both MODS and MGIT, MODS was faster than MGIT in 70%

252 (n=289/418) samples with a median time difference of 2 days (IQR: 0 – 5 days, P<0.01). In
253 smear negative samples, of 79 samples positive by both MODS and MGIT, the MODS results
254 were available 4 days earlier than MGIT (IQR: 0-7 days, P<0.01). MODS also yielded a higher
255 sensitivity than MGIT by day 7 (28% vs. 16%, P<0.001) and day 14 (57% vs. 52%, P=0.009)
256 after inoculation**.**

257 **Contamination and spoligotyping:** In total, 738 samples were cultured by both MODS and
258 MGIT. We assessed the contamination in terms of fungi or other bacteria and cross
259 contamination between samples for the MODS assay.

260 In terms of fungal contamination, the original contamination rate of MODS in samples was 1.1%
261 (n= 8/738) while it was 2.6% (n= 19/738) for MGIT. All MGIT contaminated samples were
262 decontaminated again and re-inoculated in MGIT medium. The final fungal contamination rate
263 of MGIT was 1.8% (13/738). Reprocessing for sample contaminated by MODS was not
264 attempted because of low volume (total of 1ml for each well). Contamination with fungi was also
265 observed in 8 negative control wells.

266 To assess cross-contamination of MODS with TB bacteria, spacer oligonucletide typing
267 (spoligotyping) was applied to all available MODS isolates (n=437/478). Serial positive cultures
268 from individual patients were compared for discrepancies in spoligotype. A positive MGIT
269 culture (n=41) was used for comparison if the MODS culture yielded a negative spoligotype
270 (n=21) or subculture was contaminated from MODS to LJ (n= 20). 412/437 (94%) samples had
271 defined spoligotypes while the remaining 25/437 did not because of negative MGIT culture
272 (n=3), negative spoligotype (n=3) and DNA not available (n=19). Spoligotypes were deemed

possible cross-contamination if serial isolates from an individual patient were discrepant or an isolate was H37Rv (the positive control isolate).

Eight samples from 8 patients (1.1%, n=8/738) were positive by MODS with H37Rv, the positive control strain An additional twenty-seven MODS isolates were identified as probable MODS cross-contamination due to multiple strains isolated from one patient. It is impossible to rule-out infection with multiple-strains in these patients, but the maximum cross-contamination rate of MODS with TB bacteria was 4.7% (n=35/738). All false-positive MODS cultures were in 'confirmed' or 'probable' TB groups.

Discussion

We have shown MODS to be a sensitive and rapid method for diagnosis of TB in HIV infected patients. Although MODS was slightly less sensitive than MGIT (71% vs 75%, P=0.03), MODS is faster than MGIT in samples positive by both methods with a 2 day difference (P<0.001). In smear negative TB cases, although MODS tended to be less sensitive than MGIT (38% vs 45%, P=0.078), MODS detected more cases than MGIT by day 7 (4.4% vs 0.6%, P=0.027) and day 14 (21% vs 12%, P<0.001). MODS detected 72.8% (40/55) culture-positive, smear-negative TB cases.

Therefore, MODS is an appropriate microbiological method for early detection of paucibaciliary TB; especially for HIV/TB patients.

Delays in diagnosis result in poor outcomes, increased morbidity and ongoing transmission(11). MODS detected significantly more TB cases at day 7 (4.4% vs 0.6%) and day 14 (21% vs. 12%) than commercial rapid liquid culture, similar to findings comparing MODS and Lowenstein-

Jensen in previous studies (9, 16, 21); this is crucial for early diagnosis of TB in immuno-compromised patients. Over 30% of the smear negative TB cases in our study did not receive TB treatment because the MGIT culture result was not available at discharge time. This underlies the need for a rapid diagnostic test in HIV/TB cases. Suspected TB cases who are smear negative are generally prescribed 7 to 14 days broad spectrum antibiotics to exclude other possible causes of community-acquired pneumonia before being re-tested for TB, in accordance with WHO policy (4).

Contamination is an issue with all microbiological techniques and evaluation of contamination is of importance for wide application of MODS. We have shown the fungal contamination rate to be 1.1%. Probable cross-contamination of MODS was 4.7 % which is within the expected contamination range of MGIT culture (3% to 8.5%) (14, 15, 19, 26). Cross-contamination is difficult to evaluate effectively in TB culture techniques because genotyping techniques have relatively low discriminatory power in endemic settings and it is difficult to rule-out TB infection in symptomatic patients in a high prevalence setting. The median cross contamination rate of TB laboratories is around 3% (8), but it can be much higher (24).

In conclusion, MODS is an alternative method which is rapid, sensitive, specific, cheap and feasible for the diagnosis of pauciliary TB in high burden and low resource countries.

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Figure Legends and footnotes

Figure 1 Legend. Flowchart of patient recruitment and groups of patient based on micro-confirmation (Smear or MGIT), TB treatment and outcome.

Figure 1 footnote

F/U refers to Follow-up

DTU refers to District Tuberculosis Unit

Figure 2 Legend. Time-dependent sensitivity of MODS, Smear and MGIT. The sensitivities of MODS were higher than MGIT by day 7 (P<0.001) or by day 14 (P=0.001).

Table Legends and Footnotes

Table 1 Legend: Demographic characteristics of patients.

Table 1 Footnote:

Summary measure is n (%) for all categorical characteristics.

(*)Presence of BCG scar.

P refers to the p-value of a (global) comparison of all three groups. If P<0.05, the pairwise comparisons P1, P2, P3 were also performed: P1: confirmed TB vs. probable TB, P2: Probable TB vs TB unlikely, P3: TB unlikely vs confirmed TB.

Table 2 Legend. Clinical features of 307 TB/HIV suspects.

Table 2 Footnote:

Summary measure is n (%) for all categorical characteristics.

P refers to the p-value of a (global) comparison of all three groups. If P<0.05, the pairwise comparisons P1, P2, P3 were also performed: P1: confirmed TB vs. probable TB, P2: Probable TB vs TB unlikely, P3: TB unlikely vs confirmed TB.

Table 3 Legend: . Accuracy of MODS Against microbiological confirmation as the gold standard.

Table 4 legend:. Sensitivity and Negative predictive Value (NPV) of MODS, Smear and MGIT against clinical gold standard.

Characteristic	Total population N = 307	Micro-confirmation TB N = 222	Probable TB N = 61	TB unlikely N = 24
Gender	P=1.000			
Male	301 (98.1)	217 (97.8)	60 (98.4)	24(100.0)
Age (year)	P=0.801			
Median	29	29	30	30
(IQR)	(26 – 33)	(26 – 33)	(26 – 33)	(27 – 35)
BCG vaccination(*)	P=0.858			
Yes	182 (59.3)	130 (58.7)	39 (63.9)	13 (54.2)
No	117 (38.1)	85 (38.3)	21 (34.4)	11 (45.8)
Unknown	8 (2.6)	7 (3.2)	1 (1.7)	0
TB history	P<0.001	P1=0.52	P2=0.001	P3<0.01
Yes	61 (19.9)	36 (16.2)	12 (19.7)	13 (54.2)
No	245 (79.8)	186 (83.8)	48 (78.7)	11 (45.8)
Unknown	1 (0.3)	0	1 (1.7)	0
ARV therapy	P=0.116			
Yes	42 (13.7)	29 (13.1)	10 (16.4)	3 (12.5)
No	261 (85.0)	192 (86.5)	49 (80.3)	20 (83.3)
Unknown	4 (1.3)	1 (0.5)	2 (3.3)	1 (4.2)
TB contact	P=0.758			
Yes	12 (3.9)	10 (4.5)	1 (1.6)	1 (4.2)
No	281 (91.5)	203 (91.4)	56 (91.8)	22 (91.7)

Unknown	14 (4.6)	9 (40.1)	4 (6.6)	1 (4.1)

Characteristic	Total population N = 307	Micro-confirmation TB N = 222	Probable TB N = 61	TB unlikely N = 24
History of illness				
≤ 29 days	74 (24.10)	52 (23.4)	12 (19.7)	10 (41.7)
30 – 59 days	185 (60.3)	130 (58.6)	42 (68.9)	13 (54.2)
≥ 60 days	48 (15.7)	40 (18.02)	7 (11.5)	1 (4.2)
	P=0.107			
Cough	297 (96.7) P=0.465	216 (97.30)	58 (95.1)	23 (95.8)
Fever	294 (95.8) P<0.001	216 (97.30) P1=0.63	60 (98.4) P2<0.001	18 (75.00) P3<0.001
Nightsweat	245 (79.80) P=0.106	174 (78.4)	54 (88.5)	17 (70.8)
Weightloss	292 (95.1) P=0.201	212 (95.50)	59 (96.7)	21 (87.50)
Lymphadenopathy	138 (44.9) P=0.031	106 (47.8) P1=0.08	24 (39.3) P2=0.89	8 (33.3) P3=0.25

	By patients	By samples
Sensitivity		
% (n=x/y)	87.4 (194/222)	81.0 (431/523)
95% CI	[82.3 – 95.1]	[76.3 – 85.7]
Specificity		
% (n=x/y)	93.0 (79/85)	97.0 (200/206)
95% CI	[85.3 – 97.4]	[94.8 – 99.3]
Positive predictive value		
% (n=x/y)	97.0 (194/200)	98.6 (431/437)
95% CI	[93.6 – 98.9]	[97.5 – 99.7]
Negative predictive value		
% (n=x/y)	73.8 (79/107)	66.4 (200/301)
95% CI	[64.5 – 81.9]	[58.5 – 74.4]

		MODS % (n=x/y) [95%CI]	SMEAR % (n=x/y) [95%CI]	MGIT % (n=x/y) [95%CI]	Comparison: P-value, [95%CI of difference]	
					MODS vs SMEAR	MODS vs MGIT
Sensitivity	**All subjects**					
	By patient (n=283)	71 (200/283) [64.9, 75.9]	57 (161/283) [50.9, 62.7]	75 (212/283) [69.4, 79.8]	<0.001 [8.6%, 18.9%]	0.03 [-8.1%, -0.4%]
	By sample (n=684)	64 (437/684) [58.5, 69.2]	54 (369/684) [48.2, 59.7]	70 (473/684) [63.9, 74.4]	<0.001 [6.9%, 12.9%]	<0.001 [-7.7%,-2.8%]
	Smear negative subjects					
	Patient (n=122)	38 (46/122) [29.1, 46.9]	N/A	45 (55/122) [36.1, 54.3]	N/A	0.078 [-15.4%, 0.7%]
	By sample (n=315)	29 (92/315) [22.4, 36.0]	N/A	36 (114/315) [28.8, 43.6]	N/A	0.003 [-11.5%,-2.4%]
NPV	**All subjects**					
	By patient (n=283)	22.4 (24/107) [14.9, 31.5]	16.4 (24/146) [10.8, 23.5]	25.3 (24/95) [16.9, 35.2]	0.323 [-12.7%,4.2%]	0.711 [-7.9%, 11.6%]
	By sample (n=684)	18 (54/301) [11.3, 24.6]	14.6 (54/369) [9.1, 20.2]	20.4 (54/265) [12.9, 27.8]	<0.001 [1.6%, 5.1%]	0.002 0.9, 3.9%]
	Smear negative subjects					
	By patient (n=122)	24.0 (24/100) [16.0, 33.6]	N/A	26.4 (24/91) [17.7, 36.7]	N/A	0.770 [-8.6%, 11.7%]
	By sample (n=315)	19.50 (54/277) [12.3, 26.7]	N/A	21.2 (54/255) [13.5, 28.9]	N/A	0.009 [0.42, 2.9%]