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Use of colorimetric culture methods for detection of *Mycobacterium tuberculosis* complex from sputum samples in resource-limited settings

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Running title: Colorimetric methods for tuberculosis diagnosis

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Abstract

Background: Despite recent advances, tuberculosis diagnosis remains imperfect in resource-limited setting due to complexity, cost, poor sensitivity or long time to reporting. We present a report on the use of colorimetric methods, based on the detection of mycobacterial growth using colorimetric indicators, for the detection of *Mycobacterium tuberculosis* from sputum specimens.

Methods: We evaluated the nitrate reductase assay (NRA), a modified method (NRAp) using paranitrobenzoic acid (PNB), and resazurin tube assay using PNB (RETAp) to differentiate tuberculous and non-tuberculous mycobacteria. The performances were assessed at days 18 and 28 using mycobacterium growth indicator tube (MGIT) and Löwenstein Jensen (LJ) culture methods as the reference standards.

Results: We enrolled 690 adults suspected of pulmonary tuberculosis from a regional referral hospital in Uganda between March 2010 and June 2011. At day 18 the sensitivity and specificity of NRA were 84.6% and 90.0%; 84.1% and 92.6% for NRAp; and 71.2% and 99.3% for RETAp. At day 28, the sensitivity of RETAp increased to 82.6%. Among smear-negative TB suspects, sensitivity at day 28 was 64.7% for NRA, 61.3% for NRAp and 50% for RETAp. Contamination was found in 5.4% in NRA and 6.7% in RETAp results compared to 22.1% in LJ and 20.4% in MGIT. The median times for positivity were 10, 7, and 25 days for colorimetric methods, MGIT and LJ, respectively.

Conclusion: Whereas the low specificity of NRA/NRAp precludes it from being used for TB diagnosis, RETAp could provide an alternative to LJ to accelerate time to culture results in resource-poor settings.

Introduction

Tuberculosis (TB) has a high burden in developing countries where its diagnosis mainly relies on smear microscopy (25). While simple, fast, and inexpensive, microscopy has low and variable sensitivity (20-60%) (12,12), especially among patients co-infected with HIV (11). Culture is the reference standard but is not readily available in these countries. In addition, the solid culture medium (Löwenstein-Jensen [LJ]) is too slow (3 to 8 weeks) to have an impact on patient management.

. Liquid culture mycobacterium growth indicator tube [MGIT], is more rapid (10 days) and sensitive than LJ but costly and more susceptible to contamination (28). Molecular approaches like Xpert MTB/RIF (27) reduce the time to detection (5,27) and have been endorsed by WHO (29,31). Although sensitive, rapid and relatively easy to use, these tests are costly and cannot completely replace culture, since they do not allow for the full range of drug sensitivity testing and MTB strains isolation (14,20,27).

Non-commercial alternative culture methods have been developed to reduce time and cost. Thin-layer agar (TLA) and microscopic observation drug susceptibility (MODS) assays (14,20) have shown good sensitivity and specificity compared to LJ and decreased time to detection (11.5 and 7 days, respectively). Both methods, however, require specific equipment in addition to the regular TB culture infrastructure: an inverted microscope for MODS and a CO_2 incubator for TLA (14,20). In addition, they require well-trained and experienced personnel for accurate reading.

Colorimetric methods are currently used for the rapid identification and drug sensitivity testing of *M. tuberculosis* (2,15,17,18,24). These methods rely on the detection of live bacteria through enzymatic activity. Results are obtained by direct interpretation of the

media colour and they can be performed with minimal TB culture infrastructure at relatively low cost (4).

The resazurin microtiter assay (REMA) relies on the ability of live bacteria to reduce the oxidation-reduction resazurin indicator in liquid medium (15,22,23). The nitrate reductase assay (NRA) is based on the ability of *M. tuberculosis* (MTB) to reduce nitrate in solid medium (4). Drug sensitivity results can be obtained with the two methods in 7 days from isolates to 14 days from sputum samples, with an excellent sensitivity and specificity compared to the proportion method on LJ (1,16,24). REMA and NRA are endorsed by WHO for rapid drug sensitivity testing from primary culture for colorimetric redox indicator methods, and from both smear-positive sputum or primary culture for NRA (30,31). None of these methods has been evaluated for primary detection of MTB in sputum specimen of TB suspects.

In this study, we hypothesized that these methods could be applied for primary MTB detection in sputum of pulmonary TB suspects. For the differentiation between MTB and non-tuberculous mycobacteria (NTM), we modified the RETA method with addition of an identification step using a tube containing paranitrobenzoic acid (PNB) that inhibits growth of MTB but not NTM (RETAp). Since nitrate reduction is one of the biochemical tests that are used for the differentiation of MTB and NTM, we evaluated the NRA method both with (NRAp) or without (NRA) the identification step using a PNB tube. The objective of this study was to evaluate the performance and feasibility of RETAp, NRA, NRAp for the detection of MTBC from sputum in a setting with a high prevalence of TB and HIV. We aimed to determine the performance of colorimetric assays against

LJ and manual MGIT culture in all, HIV-positive and smear-negative pulmonary TB suspects.

Material and Methods

Participants

We enrolled participants from the outpatient department and Immune Suppression Syndrome ISS (HIV) Clinic at the Mbarara Regional Referral Hospital in South-Western Uganda from March 2010 through June 2011.

Patients were eligible if they reported a cough for more than 2 weeks, were at least 15 years of age, and signed an informed consent. We excluded those with grossly bloody sputum or clear saliva, those who could not produce at least 1 mL of sputum, and those who had received anti-TB treatment for at least 1 week in the month before enrollment. The sample size of 690 patients was estimated based on the following hypotheses. We needed 96 culture-positive samples to estimate an expected sensitivity of 90% with a precision of 6%. With an expected 20% culture-positive pulmonary TB suspects, we needed to enrol 480 patients. The sample size was increased to 690 to stratify the analysis in smear-negative TB suspects who represented 80% of the overall number of TB suspects and to take into account 15% potential dropouts (8).

The study protocol was approved by the Mbarara University Faculty of Medicine Research and Ethics Committee and Institutional Ethics Committee; the Uganda National Council for Science and Technology; and the Comité de Protection des Personnes, Ile de France XI, Saint-Germain en Laye, France.

Procedures

Clinical assessment, chest X-ray, and HIV testing

All study participants underwent a physical assessment by a clinical officer and an antero-posterior chest X-ray. The study clinician recorded chest radiography findings according to a predetermined pictorial tick-sheet with final classification of the X-ray as "normal", "abnormal possible TB" and "highly suggestive of TB". Quality assurance consisted of paired interpretations of 10% of the X-rays by the hospital radiologist. HIV testing was performed for all patients who accepted after they had pre-test counselling and signed a separate informed consent. HIV testing was performed according to the national algorithm using Determine HIV-1/2/O (Abbott Laboratories, Abbott Park, IL), HIV 1/2 Stat-Pak Ultra Fast (Chembio Diagnostic Systems, Medford, NY) and Uni-Gold Recombinant HIV-1/2 (Trinity Biotech, Bray, Ireland) for discordant results between the first two tests.

Tuberculosis laboratory procedures

Microscopy and reference culture methods

We collected sputum samples on the spot and the next morning. We used auramine LED-fluorescence technique for direct microscopy on each specimen (3,6-8) and reported results according to the WHO grading scale (1). The reference standard and colorimetric methods were performed on the best quality specimen according to the laboratory technician's assessment. The specimen was then decontaminated using the NALC (0.5%)-NaOH (1.5%) method (13).

For the reference culture methods, we inoculated 100µl of decontaminated sputum into two homemade LJ tubes and 500µl into one tube of manual MGIT (Becton Dickinson, Franklin Lakes, New Jersey, USA). We reported a negative culture after 56 days of incubation. Liquefied or discoloured LJ media indicated contamination. Contamination from growth on MGIT was ruled out using Ziehl-Neelsen (ZN) microscopy and culture on blood agar. For all positive cultures on LJ and MGIT, we differentiated between MTB and NTM using the SD TB Ag MPT64 rapid system following manufacturer instructions (SD Bioline, Kyongi-do, Korea). The GenoType® Mycobacterium CM/AS Identification kit (HAIN Lifescience, Nehren Germany) was performed for identification of NTM.

Colorimetric methods

For RETAp, we inoculated 100µL of decontaminated sample into each of four 1.5-ml microtubes containing 500µL of supplemented liquid medium (Middlebrook 7H9 supplemented with oleic acid, albumin, dextrose, catalase (OADC) with 0.5% glycerol and 0.01% casitone and PANTA (Polymixin B, Amphotericin B, Nalidixic acid, thrimethoprim and Azlocillin**)** (22) and 1 microtube containing 500µL of the same medium with PNB (500µg/ml). After incubating the sample for 10 days at 37°C, we added 75µL of resazurin solution (0.01% Sigma-Aldrich, St. Louis, MO, USA) into the first microtube and incubated at 37°C overnight. If a colour change occurred from blue (oxidized state) to pink (reduced state), indicating the growth of the bacteria, we inoculated one drop on blood agar and we used one drop for ZN smear microscopy. If there was growth on blood agar and no AFB seen, we reported the tube as contaminated. We considered the result to be indeterminate if there was no growth on blood agar and no

AFB seen, or growth on blood agar and AFB seen. All positive cultures (AFB, negative blood agar) were identified as MTB or NTM by revealing the tube containing PNB with resazurin. A colour change in the tube containing PNB was considered indicative of NTM, while no colour change in the tube was considered indicative of MTB. If there was no colour change in the first tube at day 10, the other tubes were tested on days 14, 18, and 28 using the same procedure. If there was no colour change in the final tube (day 18 or day 28) the sample was considered negative. If the final tube was contaminated, then the sample was considered to be contaminated.

For NRA, we inoculated 200µL of decontaminated sample into four tubes of LJ media containing a final concentration of 1 g/L of potassium nitrate (Sigma-Aldrich, St. Louis, MO, USA) and one tube of LJ media containing the same concentration of potassium nitrate with PNB (500µg/ml). After incubating the samples for 10 days at 37°C, we added 500µl of fresh reagent mix (one part 50% concentrated hydrochloric acid, two parts 0.2% sulfanilamide, and two parts 0.1% N-(1-Naphthyl)ethylenediamine dihydrochloride) into the first tube. If the colour of the reagent mix changed to purple immediately after addition of the reagent in this tube, we considered it to be positive. For the NRA assay interpretation, a positive result was considered positive for MTB. For the NRAp assay, the NRA+PNB tube was revealed similarly and the test was considered positive for MTBC if there was no colour change and positive for NTM if there was a colour change. Culture results were interpreted in isolation from all other clinical and laboratory results.

Quality control and quality assessment

We randomly selected 10% of all sputum smear slides and all positive or scanty result slides that were read in duplicate for internal quality control. Discrepancies were resolved by the TB laboratory supervisor. Our laboratory participates in a proficiency scheme with the National Health Laboratory Services in South Africa for external quality control of TB culture and smear-microscopy.

Statistical analysis

Data were double entered using Voozanoo software (EpiConcept, Paris France). Statistical analysis was performed using Stata SE v.11 software (College Station, Texas, USA).

We considered a sample positive by the gold standard if MTBC was isolated from either LJ or MGIT. We considered a sample negative if both were negative, or if one medium was negative and the other contaminated. For each colorimetric assay, performance was calculated by estimating the sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, and their 95% confidence intervals. Contaminated and positive culture results for NTM by either the reference standard or the tested culture methods were excluded from the analysis of performance. The performances of NRA and RETAp were estimated separately after 18 and 28 days of incubation. Analysis was performed in all smear-negative and HIV-infected pulmonary TB suspects. In a secondary analysis, we compared the MTB recovery rate between RETAp and LJ among MGIT-positive cases using a McNemar test. We described the

median time with inter-quartile range to culture positivity of the different culture methods.

For the feasibility assessment, after the conclusion of the study, laboratory technicians were asked to complete an ease of use questionnaire on the culture media preparation, specimen processing and reading of the results. We calculated the costs of reagents and materials for the different methods.

Results

Participants

We screened 1,071 TB suspects and 671 (62.7%) patients in the final analysis (Figure 1). Participant characteristics are presented in Table 1. Of these 671 patients, 590 patients (87.9%) provided two specimens. The specimen selected for the study was the early morning specimen in 529 (78.8%) subjects. The macroscopic appearance was purulent-mucopurulent in 212 (31.6%), mucoid in 446 (66.5%), blood-stained in 2 (0.3%), salivary in 2 (0.3%) and unknown in 9 (1.3%) samples. We detected 108 patients (16.1%) with a positive smear result among the 671 patients included in the analysis.

Reference culture results

Out of the 671 patients included in the analysis, LJ was MTB positive in 107 (16.0 %), NTM positive in 4 (0.06%), negative in 416 (62.0 %), and contaminated in 148 (22.1 %). MGIT was MTB positive in 142 (21.2 %), NTM positive in 8 (1.2%), negative in 392 (58.4 %) and contaminated in 137 (20.4 %). When combined, 46 (6.9%) and 8 (1.2%)

patients had a final contaminated culture and NTM result, respectively. After exclusion of contaminated and NTM results, the reference standard was positive in 148 (23.7%). The proportion of MTBC culture positive results by the reference method were 6.2% (n=35/563) among smear-negative and 21.8% (73/335) among HIV-infected patients.

Colorimetric assay results

The results of NRA, NRAp, and RETAp after 18 days of incubation cross-tabulated with the reference culture results are shown in Table 2. At day 28 compared to day 18, an additional 19 MTBC were identified by RETAp, for an incremental yield of 18.1% (19/105), and 13 by NRA or NRAp, for an incremental yield of 8.7% (13/149) for NRA and 7.5% (13/174) for NRAp (Table 3). Accordingly, the sensitivity of the three methods increased between day 18 and 28, while specificity slightly decreased (Tables 4 and 5). Sensitivities were lower in smear-negative and in HIV-infected patients than in all TB suspects (Tables 4 and 5).

There were 53 false positive results by the NRA methods, among which NRAp classified 13 as NTM. Among the 40 patients classified as MTBC by NRAp, chest radiography was classified as possible or highly suggestive of TB in 45.5% (15/33); 73.0% of patients (27/37) were HIV positive; and one was smear positive (2.5%). Both reference cultures were negative for 19 (47.5%) of the false positive NRAp specimens, while one was negative and the other contaminated for the remaining 21 (52.5%).

Comparison between LJ and RETAp

Of the 142 specimens positive by MGIT, 101 (71%) were positive by LJ and 111 (78%) by RETAp at day 28 (p=0.099) but there were 20 (14%) LJ contaminated results compared to 0 (0%) for RETAp.

Time to positivity

The median times for positivity (IQR) were 10 days (10-14) for NRA, 10 days (10-14) for NRAp, and 10 days (10-18) for RETAp. Comparatively, the median times for positivity were 7 days (4-11) for MGIT and 25 (21-35) days for LJ. The NRA/NRAp detected 93.1% and RETAp detected 84.8% of positive culture specimens by day 18 of incubation (Figure 2).

Feasibility and cost

Each medium was prepared locally, stored at room temperature, and cost less than 1 USD for RETAp or NRA. The analysis of one sample cost 0.5 and 10 USD for LJ and MGIT respectively. Laboratory technicians reported high acceptability and feasibility of the colorimetric assays.

DISCUSSION

We present the first report on the use of colorimetric methods for primary detection of MTB for diagnosis of pulmonary TB. These assays were simple to use, showed low contamination levels with relatively short time to positivity results compared to standard culture methods; however, they had disappointing performances.

274 The NRA method showed acceptable sensitivity overall (84.6%) but had low specificity
275 (90%). The addition of PNB in the NRAp method slightly increased the specificity to
276 93%. The low specificity could be due to false positive results caused by contaminants
277 such as *Pseudomonas spp* or *Staphylococcus spp* that can cause nitrate reduction (10,26).
278 This may also partly explain the lower contamination rate observed for NRA (6.7%)
279 compared to LJ (22.1%). An additional step of ZN microscopy and/or MTB identification
280 rapid test on positive samples might improve NRA specificity.
281 In contrast, RETAp had excellent specificity (99.3%), but its sensitivity (71.2%) was
282 relatively low when read at day 18, especially among smear-negative patients (50%).
283 Extending detection to 28 days instead of 18 days increased the sensitivity to 82.6% and
284 an additional reading after 28 days should be systematically planned for negative RETAp
285 samples.
286
287 The performances of the colorimetric methods for primary MTB detection seem to be
288 slightly lower than the performances of MODS and TLA from a recent systematic review
289 with a sensitivity of 92% (95%CI 87–97) for MODS and 87% (95%CI 79–94) for TLA
290 and a specificity of 96% (90–100) for MODS and 98% (95%CI 94– 100) for TLA (14) .
291 However, the performances of RETAp after 28 days are comparable with those of MODS
292 in studies including both smear-positive and smear-negative specimens, and using liquid
293 and solid cultures as a reference standard (14,20). Nevertheless, compared to these other
294 non-commercial assays, the colorimetric methods provide important advantages in terms
295 of ease of interpretation, because they rely on a colour change instead of observation of
296 microcolonies under the microscope (19-21), and as such, are probably much less prone

297 to inter-user variability. Comparison of the accuracy, inter-user reproducibility, and time
298 to detection between these non-commercial rapid cultures would be helpful.
299
300 LJ remains the most used culture method for diagnosing TB in developing countries,
301 although it is less sensitive than MGIT (9). In this first study on colorimetric methods for
302 MTB detection, RETAp MTB recovery results seem to be comparable to LJ. However,
303 the LJ positive detection rate might be underestimated because of the high proportion of
304 LJ contaminated results, which represent 14.2% of the positive results by MGIT
305 compared to 0% with RETAp. The lower contamination of RETAp could be due to the
306 addition of PANTA in the tube as well as the use of four tubes for revelation of RETAp
307 compared to two tubes for LJ. These results should be first confirmed in another study,
308 but they are encouraging taking into consideration the short time to positivity of RETAp
309 compared to LJ (median 10 versus 25 days).
310
311 The performances of RETAp at day 28 are slightly lower than performances of the Xpert
312 MTB/RIF reported in numerous studies on this new method (Cochrane review's pooled
313 sensitivity 88% [95% CI 83-92) overall; 68% [95% CI 59-75) among smear-negative
314 patients; 76% [95% CI 63-85) among HIV-positive patients. Culture methods in general
315 cannot compare with this molecular test in terms of time to obtain results and simplicity
316 of use. The main advantages of culture methods over Xpert are that they can test for
317 susceptibility to multiple drugs as opposed to rifampicin alone (5) and they allow the
318 storage of cultured strains for quality control and further investigations. Culture methods
319 are also more affordable for settings with existing culture-testing capacity: Xpert
320 MTB/RIF costs 10 USD compared to the home-made colorimetric media (<1 USD per

specimen). However, in contrast to Xpert MTB/RIF, all other culture methods do require a P2/P3 level of safety equipment for the culture of MTB.

Our study was limited by the lower than expected number of smear-negative and culture-positive specimens in our sample, which resulted in wide confidence intervals for analyses in this sub-group. This is due to an overestimation of the MTB growth in specimens of smear-negative pulmonary TB suspects based on a previous report of 10-20% in the region (6-8). Another limitation was the high contamination rate of LJ and MGIT media. Despite the high quality of the standard laboratory procedures, as confirmed by external monitors from the supranational reference laboratory at the Institute of Tropical Medicine (Antwerp, Belgium), contamination rates remained high throughout the study (22%). A possible explanation is that our broad inclusion criterion, defined as cough lasting more than 2 weeks, might have led to inclusion of patients with diseases other than TB, caused by pathogens such as *Pseudomonas spp* or *Staphylococcus spp* that are known to be resistant to our decontamination process.

CONCLUSION

In conclusion, this first evaluation of the colorimetric methods as non-commercial alternatives to MGIT for rapid MTB culture-based detection shows disappointing results in terms of performance. The low specificity of NRA, even after addition of PNB, precludes it from being used for primary MTB detection. However, RETAp may have a place in laboratories already using LJ to speed up culture results and potentially reduce culture contamination. In addition, the ease of reading of the results is an advantage

344 compared to other non-commercial culture methods. Further evaluation of the RETAp for
345 diagnosis of TB in comparison with other non-commercial culture methods would be
346 necessary.
347

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465 and technical advisory group for tuberculosis. WHO, Geneva . 2010.
466 Ref Type: Generic
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470 **Table 1. Baseline characteristics of patients included in the study (N=671 if not specified otherwise)**

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Characteristics	Result
Median age in years median (IQR)	38 [30, 48]
Gender ratio (M:F)	49:51
Patients referred by n (%)	
OPD	392 (58.4)
ISS clinic	257 (38.3)
Other	22 (3.3)
HIV-positive n (%) (N=637)	373 (58.6)
Clinical signs at inclusion n (%)	
History of cough (N=671)	671(100.0)
History of fever >2 weeks (N=670)	258 (38.5)
Chest pain (N=663)	482 (72.7)
Haemoptysis (N=664)	78 (11.8)
Night sweats (N=663)	102 (15.4)
Reported weight loss (N=663)	488 (73.6)
Treatment history n (%) (N=654)	
At least one medication in previous 30 days	654 (100.0)
Antibiotics	425 (65.0)
Cotrimoxazole	329 (50.3)
Amoxicillin and derivatives	140 (21.4)
Anti-TB drugs > 1 month before inclusion	17 (2.6)
Previous Antiretroviral treatment	176 (26.9)
Antiretroviral treatment at inclusion	81 (12.4)
Chest X-ray Outcomes n (%) (N=611)	
No TB	259 (42.5)
Maybe TB	215 (35.4)
Definitely TB	135 (22.1)

472 **Table 2. Results of the colorimetric assays at Day 18 by results of the reference standard (N=671)**

Colorimetric assays	Reference standard			
	Negative	MTBC	NTM	Contaminated
NRA				
Negative	399	21	6	26
Positive	45	115	1	13
Contaminated	33	4	1	7
NRAp				
Negative	399	21	6	26
MTBC	32	111	0	6
NTM	13	4	1	7
Contaminated	33	4	1	7
RETAp				
Negative	450	40	6	30
MTBC	3	99	0	0
NTM	3	0	1	0
Indeterminate	2	0	0	1
Contaminated	19	1	1	15

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476 **Table 3. Results of the colorimetric assays at Day 28 by results of the reference standard (N=671)**

Colorimetric assays	Reference standard			
	Negative	MTBC	NTM	Contaminated
NRA				
Negative	392	19	6	24
Positive	53	118	1	15
Contaminated	32	3	1	7
NRAp				
Negative	392	19	6	24
MTBC	40	114	0	8
NTM	13	4	1	7
Contaminated	32	3	1	7
RETAp				
Negative	448	24	7	27
MTBC	5	114	0	2
NTM	3	0	1	0
Indeterminate	1	1	0	1
Contaminated	19	1	0	16

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478 **Table 4. Performance of colorimetric methods in all TB suspects, smear-negative, or HIV-positive**

479 **patients at Day 18**

		Sensitivity [95% CI]	Specificity [95% CI]	PPV [95% CI]	NPV [95% CI]	LR+ [95% CI]	LR- [95% CI]
NRA							
	All TB suspects	84.6 [77.4-90.2]	90.0 [86.7-92.5]	71.9 [64.2-78.7]	95.0 [92.5-96.9]	8 [6-11]	0.17 [0.12-0.25]
	Smear-negative	61.8 [43.6-77.8]	90.0 [86.9-92.7]	32.3 [21.2-45.1]	96.8 [94.7-98.3]	6 [4-9]	0.42 [0.28-0.65]
	HIV-positive	78.1 [66.9-86.9]	88.5 [84.0-92.1]	65.5 [54.6-75.4]	93.5 [89.7-96.3]	7 [5-10]	0.25 [0.16-0.38]
NRAp							
	All TB suspects	84.1 [76.7-89.9]	92.6 [89.7-94.9]	77.6 [69.9-84.2]	95.0 [92.5-96.9]	11 [8-16]	0.17 [0.12-0.25]
	Smear-negative	58.1 [39.1-75.5]	92.8 [89.9-95.0]	36.7 [23.4-51.7]	96.8 [94.7-98.3]	8 [5-12]	0.45 [0.30-0.68]
	HIV-positive	77.5 [66.0-86.5]	91.7 [87.5-94.8]	72.4 [60.9-82.0]	93.5 [89.7-96.3]	9 [6-14]	0.25 [0.16-0.38]
RETAp							
	All TB suspects	71.2 [62.9-78.6]	99.3 [98.1-99.9]	97.1 [91.6-99.4]	91.8 [89.0-94.1]	107 [35-334]	0.29 [0.22-0.38]
	Smear-negative	25.7 [12.5-43.3]	99.3 [98.1-99.9]	75.0 [42.8-94.5]	94.5 [92.1-96.4]	39 [11-136]	0.75 [0.62-0.91]
	HIV-positive	63.0 [50.9-74.0]	98.8 [96.6-99.8]	93.9 [83.1-98.7]	90.4 [86.3-93.5]	54 [17-168]	0.37 [0.28-0.51]

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481 **Table 5. Performance of colorimetric methods in all TB suspects, smear-negative and HIV-positive**

482 **patients at Day 28**

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		Sensitivity [95% CI]	Specificity [95% CI]	PPV [95% CI]	NPV [95% CI]	LR+ [95% CI]	LR- [95% CI]
NRA							
	All TB suspects	86.1 [79.2-91.4]	88.1 [84.7-90.9]	69.0 [61.5-75.8]	95.4 [92.9-97.2]	7 [6-9]	0.16 [0.10-0.24]
	Smear-negative	64.7 [46.5-80.3]	88.3 [84.8-91.1]	29.7 [19.7-41.5]	97.0 [94.9-98.5]	6 [4-8]	0.40 [0.25-0.63]
	HIV-positive	78.1 [66.9-86.9]	88.5 [84.0-92.1]	65.5 [54.6-75.4]	93.5 [89.7-96.3]	7 [5-10]	0.25 [0.16-0.38]
NRAp							
	All TB suspects	85.7 [78.6-91.2]	90.7 [87.6-93.2]	74.0 [66.4-80.8]	95.4 [92.9-97.2]	9 [7-13]	0.16 [0.10-0.24]
	Smear-negative	61.3 [42.2-78.2]	90.9 [87.8-93.5]	32.8 [21.0-46.3]	97.0 [94.9-98.5]	7 [4-10]	0.43 [0.27-0.66]
	HIV-positive	80.3 [69.1–88.8]	89.3 [84.9-92.8]	67.9 [56.8-77.6]]	94.2 [90.4-96.8]	8 [5-11]	0.22 [0.14-0.35]
RETAp							
	All TB suspects	82.6 [75.2-88.5]	98.9 [97.4-99.6]	95.8 [90.5-98.6]	94.9 [93.5-96.7]	75 [31-180]	0.18 [0.12-0.25]
	Smear-negative	50.0 [32.4-67.6]	98.9 [97.4-99.6]	77.3 [54.6-92.2]	96.3 [94.2-97.8]	45 [17-115]	0.51 [0.36-0.71]
	HIV-positive	77.8 [66.4-86.7]	98.0 [95.5-99.4]	91.8 [81.9-97.3]	94.0 [90.5-96.5]	40 [17-96]	0.23 [0.15-0.35]

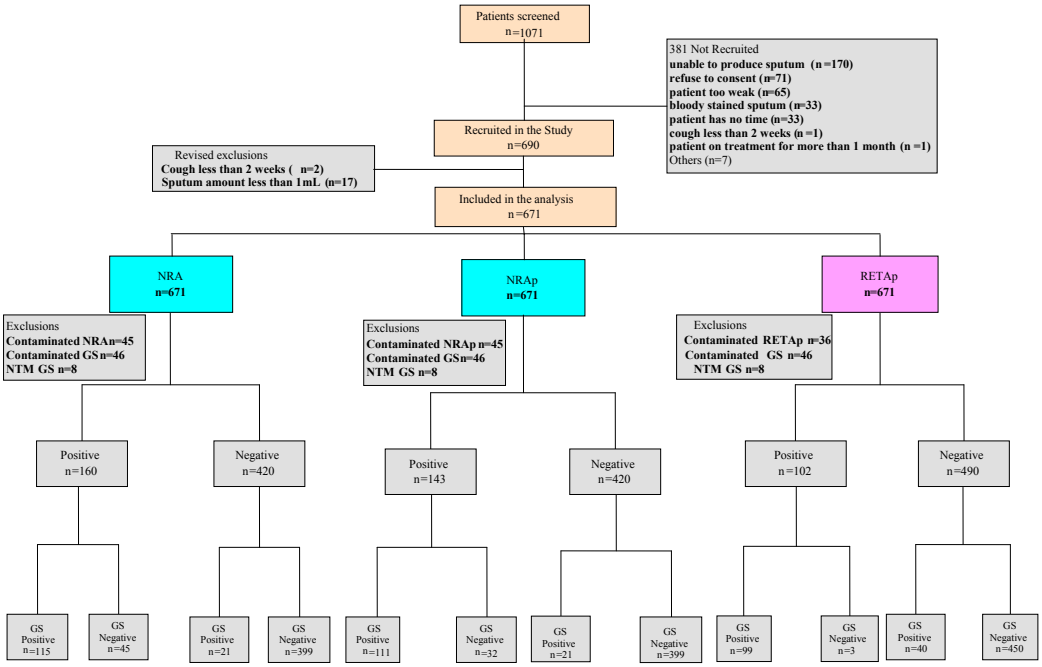
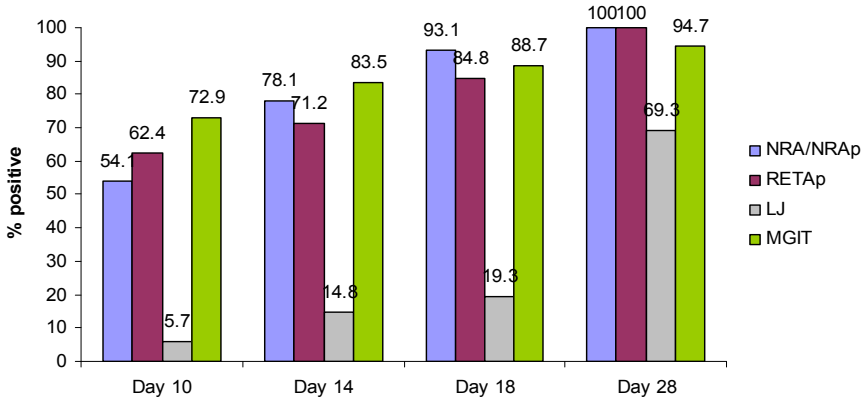


Figure 1. Recruitment of patients and culture results



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487 **Figure 2. Cumulative percentage of positive specimens at Day 10, 14, 18 and 28 for each medium**