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Sputum induction is a safe procedure to use in prisoners and MGIT is the best culture method to diagnose tuberculosis in prisons: a cohort study

Zulma Vanessa Rueda [a,*], Lucelly López [b], Diana Marín [c], Lázaro A. Vélez [a], María Patricia Arbeláez [d]

[a] Grupo Investigador de Problemas en Enfermedades Infecciosas, Facultad de Medicina, Calle 62 # 52-59, lab 630, Sede de Investigación Universitaria, Universidad de Antioquia, Medellín, Colombia
[b] Facultad de Medicina, Universidad Pontificia Bolivariana, Medellín, Colombia
[c] Grupo Demografía y Salud, Facultad Nacional de Salud Pública, Universidad de Antioquia, Medellín, Colombia
[d] Grupo de Epidemiología, Facultad Nacional de Salud Pública, Universidad de Antioquia, Medellín, Colombia

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SUMMARY

Objectives: To evaluate the concordance and safety of induced sputum (IS) and spontaneous sputum (SS), and estimate concordance and time to detection of *M. tuberculosis* between Lowenstein–Jensen (LJ), thin-layer agar (TLA), and the Mycobacteria Growth Indicator Tube system (MGIT).
Methods: This was a cohort study. Prisoners with pulmonary tuberculosis (PTB) were followed for 2 years. At baseline and every follow-up visit, three sputum samples were taken on consecutive days (one IS and two SS) and adverse events occurring before, during, and 30 min after IS were registered. All sputum samples were stained with auramine and cultured in LJ, TLA (to test resistance), and MGIT.
Results: Five hundred eighty-six IS and 532 SS were performed on 64 PTB patients. Breathlessness (1.6%), cough (1.2%), hemoptysis (0.3%), and cyanosis (0.2%) were the only complications. Concordance between IS and SS was 0.78 (95% confidence interval 0.69–0.87); 11 positive cultures from IS samples were negative in SS, and 11 positive cultures from SS samples were negative in IS. One hundred seventy-eight cultures were positive by any technique: MGIT 95%, LJ 73%, and TLA 57%. Time to detection of *M. tuberculosis* in LJ, TLA, and MGIT was 31, 18, and 11 days, respectively.
Conclusions: The IS procedure is safe in prisons. The MGIT system is better and faster than LJ and TLA in the diagnosis of *M. tuberculosis*.

1. Introduction

Tuberculosis (TB) is a public health threat, particularly in prisons where the incidence of TB and latent TB infection is high.[1] The second element of the directly observed therapy strategy (DOTS) is case detection through quality-assured bacteriology, and includes bacteriology for diagnosis (sputum smear microscopy, culture, and drug susceptibility testing) and strengthened laboratory networks.

In Colombia, guidelines published by the Ministry of Health state that culture of a sputum sample is mandatory for prisoners with suspected TB.[2] This policy is not adhered to due to the lack of a well-implemented TB program in the country and, with regard to prisons, a lack of political commitment, inadequate financing for diagnostic laboratories, and a lack of awareness of the magnitude of TB incidence in prisons. This situation is similar in other prisons around the world.[3]

Sputum smear microscopy remains the cornerstone of TB diagnosis in developing countries and in prisons; however, it is hampered by low sensitivity ranging from 50% to 80% for active pulmonary TB cases, decreasing to 20% in HIV-infected individuals. There must be 5000 to 10 000 bacilli per milliliter of specimen to allow the detection of bacteria in stained smears, whereas culture is able to detect as few as 10 bacteria per milliliter.[4] Also, there are factors that influence the sensitivity, such as the staining technique, centrifugation speed, reader experience, and the prevalence of TB in the population being tested.[4] The inadequate sensitivity of sputum smear microscopy leads to misdiagnosis in

* Corresponding author. Tel.: +57 4 2196541.
E-mail address: zulmaruedav@gmail.com (Z.V. Rueda).

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up to a half of patients with active TB, which means that, on average, a person with active TB will infect between 10 and 15 people every year unless he/she receives adequate treatment. Furthermore, overcrowding and poor ventilation in prisons provide optimal conditions for the transmission of TB. Therefore, it is important to use culture. However, conventional cultures (Ogawa–Kudoh and Lowenstein–Jensen (LJ)) require a protracted incubation period (mean range from 25.6 to 32.6 ± 11.8 days[5–10]) due to the slow growth rate of TB bacilli. A good alternative to decrease the time to detection of *Mycobacterium tuberculosis* is the use of liquid cultures. In Colombia, there are two alternatives: the Mycobacteria Growth Indicator Tube system (MGIT) and thin-layer agar (TLA). These methods have been shown to decrease the mean time to detection of growth to 11–15.1 days.[5,6,9,11–14]

The method of obtaining a respiratory sample is also critical. In prisons, it is important to employ alternative strategies to obtain a specimen when the patient cannot expectorate or has inadequate sputum (HIV patients or those with a dry cough), during follow-up to confirm that the patient is cured, or for people who are sputum smear-negative. Although bronchoscopy for bronchoalveolar lavage (BAL) is an alternative, this procedure is invasive, expensive, and requires specialized personnel and equipment that is not available in prisons. The lack of available resources necessitates the transport of prisoners to a hospital in order to obtain a BAL sample, which may not be feasible due to safety issues or limited resources. Sputum induction could, therefore, be an option in prisons. Recent studies have reported that the overall success of sputum induction is high (76.4–100%), while adverse events associated with induced sputum (IS) are infrequent and mild.[15] Also, the diagnostic yield of IS has been found to range from 35% to 95% and there are no differences in the yield according to HIV prevalence or age.[16]

The objectives of this study were (1) to evaluate the safety of the IS procedure; (2) to compare the quality of IS and spontaneous sputum (SS) samples and estimate the concordance between them; and (3) to estimate the concordance and the time to detection of *M. tuberculosis* in three cultures: LJ, TLA, and MGIT.

2. Materials and methods

2.1. Study design and setting

This prospective cohort study was carried out in four prisons (two male and two female) in two cities in Colombia (Medellín and Bucaramanga). These prisons have middle and high security block cells, and there are sentenced individuals and others awaiting sentencing. The percentage of overcrowding in three of the prisons is 147.5%, 95%, and 33%, respectively.[17] One prison was recently constructed and divided into male and female prisons and there is no overcrowding at present.

2.2. Participants, procedures, and follow-up

All prisoners older than 18 years of age and diagnosed with active pulmonary TB were eligible for inclusion in the study.

People diagnosed with active pulmonary TB by sputum smear or culture were followed for 2 years or until the end of the study, from April 30, 2010 to December 23, 2012 in Medellín, and from April 30, 2010 to June 30, 2011 in Bucaramanga, as described previously.[17] Follow-up visits were done monthly for 6 months after starting anti-TB treatment, or 9 months in the setting of HIV or monoresistance. Subsequent follow-up was carried out on a bimonthly basis for the next 6 months, and quarterly during the second year. During each follow-up, one sample of SS was taken followed by one sample of IS an hour after the SS on the first day, and one SS sample was taken the next day.

The day prior to sputum induction, patients were instructed not to brush their teeth with toothpaste before the procedure. Each patient received detailed information and clear instructions about the sputum induction. Patients who agreed to the procedure were asked about any contraindication to the procedure, such as a history of massive epistaxis necessitating an emergency room visit, history of bleeding disorders, history of heart failure, chest tube drainage for pneumothorax, recent eye surgery, and history of severe asthma requiring treatment in the intensive care unit. In the absence of a contraindication, participants were asked for the presence of the following symptoms in the last 48 h: persistent cough, hemoptysis, dyspnea, and pleuritic chest pain. The same symptoms were sought during and 30 min after the IS procedure. A physical examination was performed before, during, and after the IS procedure. Due to the lack of negative pressure isolation rooms in prisons, the procedures were conducted in sports fields within the prisons. These places were chosen because they are far from other individuals and they are well-ventilated areas.

The procedure was carried out by trained personnel (a physician and a nurse or a physical therapist). During sputum induction, the personnel kept a distance of 5 m from the participant, while observing the patient at all times. Supplemental oxygen and full resuscitation were available during the procedure. Before sputum induction, the patients rinsed their mouth with water. The field team instructed patients to spit into the container when they felt the urge to cough. Pretreatment with salbutamol 200 μg (two puffs from a standard metered-dose inhaler) was given. The nebulization started with hypertonic saline solution (5%) 15–20 min after bronchodilator pretreatment using a model 1121 MEDI-PUMP nebulizer. The field team stopped the procedure 10 min after the nebulization was started to perform a physical examination, after which nebulization was restarted until the patient completed 15 to 20 min. All sputum samples were taken under direct supervision by the field team.

All sputum samples were processed using the conventional sodium hydroxide–*N*-acetyl-L-cysteine method, decontamination, and concentration methods. A smear was prepared for auramine–rhodamine staining to visualize acid-fast bacilli (AFB). The first sample of SS and the IS sample of each person was inoculated in LJ medium, in MGIT incubated in an MGIT 960 BACTEC instrument (BD Diagnostics, Sparks, MD, USA), and in TLA for the detection of resistance to rifampicin and isoniazid, as reported previously.[18] *M. tuberculosis* was identified by standard biochemical tests. All procedures were done as described previously.[18–20] All contaminated cultures for all three media (LJ, TLA, and MGIT) were considered negative.

2.3. Study variables

The following information was collected: persistent cough, hemoptysis, dyspnea, pleuritic chest pain, heart rate, blood pressure, respiratory rate, temperature, breathlessness, abnormal breath sounds on lung auscultation, epistaxis, and cyanosis. The consistency, presence of mucus and blood, and volume of sputum samples were recorded. The presence of *M. tuberculosis* in each culture, the date of TB treatment initiation, the date on which the sputum samples were taken, and the date when each culture was positive were also noted.

2.4. Definitions

A patient without any clinical signs before sputum induction, who presented breathlessness, cyanosis, hemoptysis, or cough during and/or after the sputum induction, was considered to have experienced an adverse event.

The percentage concordance between IS and SS was considered when the results were the same in both IS and SS for each category of consistency, presence of mucus, presence of blood, and volume.

The quality of IS was considered to be better than that of SS when IS had better characteristics than SS for each category related to the quality of the sputum sample; for example, when the IS had a thick consistency and SS was saliva. The quality of IS was considered to be worse than SS when IS had worse characteristics than SS for each category of quality of the sputum sample.

2.5. Statistical analysis

Data were entered into a Microsoft Access database and the statistical analysis was performed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA) and STATA 11.1 (StataCorp, College Station, TX, USA).

Although we followed all patients with diagnosed TB, a person with a positive sputum smear but negative culture for *M. tuberculosis* was excluded from this analysis, as were individuals with missing data.

To evaluate the safety of IS, we included all IS procedures and described the clinical signs that patients had before sputum induction. Then, the percentage of adverse events associated with the procedure (during and after) in TB patients was estimated.

To compare the quality of sputum samples between SS and IS, all patients who had both sputum samples at diagnosis or at each follow-up were included in the analysis. The concordance and the 95% confidence interval (95% CI) of the quality of the sample between SS and IS and the culture results obtained by SS and IS were estimated using the Kappa coefficient. The agreement for SS and IS was interpreted as poor when the calculated values were between 0 and 0.4, moderate between 0.4 and 0.6, good between 0.6 and 0.8, and excellent >0.8. Negative values were interpreted as equal to 0.0. The diagnostic yield of IS was estimated as the number of positive cultures by IS divided by the total number of positive cultures by both IS and SS, multiplied by 100.

A Venn diagram was used to show the agreement of positive cultures obtained by each technique (LJ, TLA, and MGIT). The median (interquartile range) of the time to detection of *M. tuberculosis* in each culture was estimated at diagnosis and during TB treatment. Finally, a generalized linear mixed model was used to estimate the difference of means of the time to detection of *M. tuberculosis* between cultures (LJ, TLA, and MGIT), and the Wald Chi-square statistic and *p*-value were reported. The dependent variable was the time to detection of *M. tuberculosis*, the independent variable was the culture technique, and the time variable was each month of follow-up after TB treatment initiation.

3. Results

Of 72 patients diagnosed with TB, seven were excluded from the analysis of this study (Figure 1). There were 632 follow-up procedures for 64 patients with a positive sputum culture. Among these patients, four refused sputum induction and four patients did not provide the SS during follow-up. In addition, three patients withdrew during follow-up (at months 1, 2, and 21). Nineteen patients were lost to follow-up (one at month 4, three at the month 5, two at month 8, two at month 10, three at month 15, four at month 18, three at month 21, and one at month 24). In the end, there were 586 IS samples and 532 SS samples (Figure 1).

3.1. Safety of sputum induction

In 159 episodes (of the 573 IS procedures), the patients had a cough before the procedure, nine had breathlessness, two cyanosis, and one had hemoptysis. Among them, five continued to cough and five continued to experience breathlessness after the procedure.

The adverse events associated with IS were as follows: 1.6% (9/562) had breathlessness during and after the procedure, 1.2% (5/414) had a cough, 0.3% (2/572) had hemoptysis, and 0.2% (1/571) had cyanosis. There were no severe complications during or after the procedure.

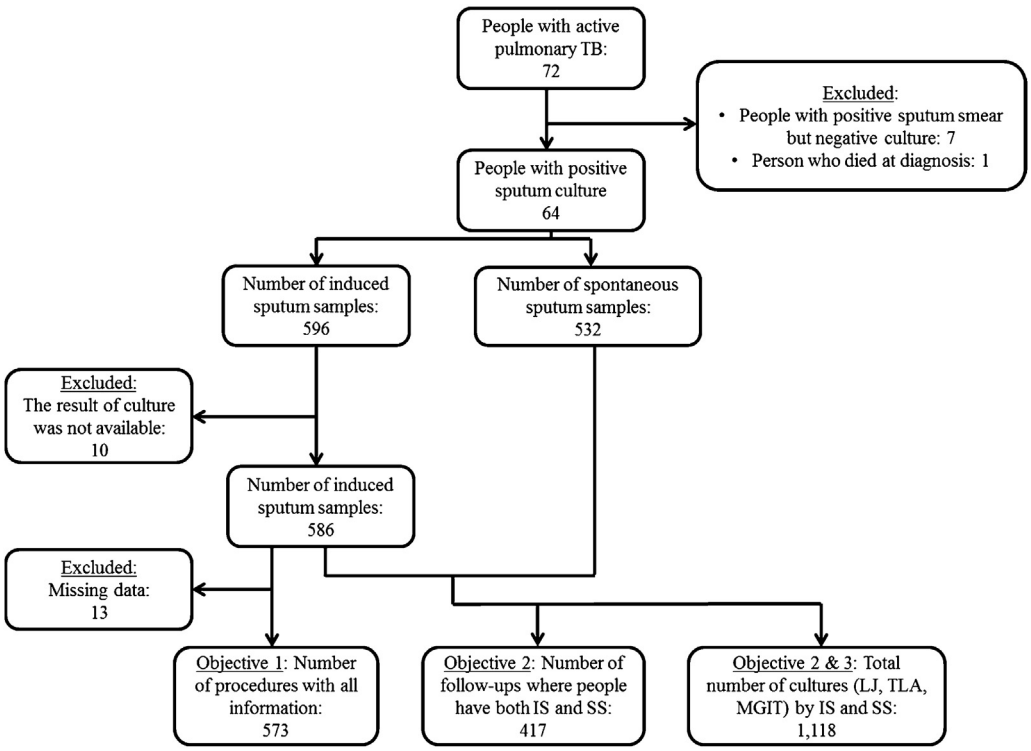

Figure 1. Flowchart of the analysis for this study.

3.2. Spontaneous versus induced sputum

Of 632 follow-ups, there were 417 at which individuals had both IS and SS. The percentage of discordance between IS and SS was similar with regards to consistency (14.2% vs. 11.0%) and the presence of mucus (26.1% vs. 23.7%) and blood (0.5% vs. 0.3%), but IS provided a larger specimen volume (40.2% vs. 18.1%) compared to SS (Table 1).

Results for the concordance between IS and SS for consistency, presence of mucus, presence of blood, and volume are given in the **Supplementary Material** (Table S1).

The concordance between the positive and negative cultures obtained by IS and SS was 0.78 (95% CI 0.69–0.87) (Table 2). The diagnostic yield of IS was 84.1% (58/69) (95% CI 74.7–93.4%).

The 11 cultures that were positive by IS but negative by SS, were positive as follows: one at diagnosis, four at month 1, four at month 2, one at month 3, and one at month 4. The other 11 cultures that were positive by SS, but negative by IS were positive as follows: five at diagnosis, one at the month 1, three at month 2, and two at month 3.

There were no differences between the yield of IS and SS by sex or HIV status.

3.3. Culture techniques and time to detection of M. tuberculosis

Among 1118 cultures (586 IS and 532 SS) obtained at diagnosis and during follow-up (Figure 1), there were 178 positive cultures by at least one technique. Among them, MGIT was positive in 170 cultures (95.5%), LJ in 130 (73%), and TLA in 102 (57%). All together LJ, TLA, and MGIT were positive in 86 cultures (Figure 2).

At diagnosis and during follow-up, MGIT culture was most likely to diagnose a TB case and had the shortest time to detection of *M. tuberculosis* (Figure 3) compared to LJ and TLA; this was statistically significant (p-value <0.0001, Wald Chi-square statistic 284.6, degrees of freedom = 2).

In addition, MGIT culture had similar results by AFB smear-negative and smear-positive sputum results (Table 3).

The number of positive cultures by both IS and SS at TB treatment initiation and follow-up, and the median and inter-quartile range of the time to detection of *M. tuberculosis* in LJ, TLA, and MGIT are given in the **Supplementary Material** (Table S2).

4. Discussion

The main findings of this study were the following: (1) IS is a safe procedure and can be implemented in the prison setting, when SS is not obtainable and during follow-up; (2) IS and SS have similar sample quality characteristics in terms of consistency, presence of mucus, and absence of blood, but the volume of sputum is higher in IS compared to SS; (3) the concordance between IS and SS is good, although the SS sample identified more cases at diagnosis, while IS had more positive cultures during follow-up; and (4) the liquid medium (MGIT) had the highest percentage of positive cultures compared to LJ and TLA, and reduced the time to detection of *M. tuberculosis*.

This study shows that IS can be used safely in prisons as it has mild and infrequent adverse events. This finding is similar to those of other studies in adults, which have reported mild symptoms such as cough, epistaxis, nausea, and vomiting,[21–23] or no adverse events.[24–26] IS is relatively easy to obtain, even in the suboptimal prison setting.

The specimen quality of IS is equivalent to SS. In a systematic review and meta-analysis on sputum induction for the diagnosis of pulmonary TB by Gonzalez-Angulo et al., all 17 studies included were cross-sectional and there was substantial heterogeneity in saline concentrations (ranging from 3% to 20%), a variety of ultrasonic nebulizers or conventional air compressors were used, and the duration of saline nebulization varied. Also, the studies compared IS with a different method – gastric lavage, fiberoptic bronchoscopy (FOB), SS, string test, lymph node biopsy, and nasopharyngeal aspiration. The diagnostic yield for IS ranged from 35% to 95%, with a pooled diagnostic yield in adults of 71% (95% CI 60–81%), and there was no difference according to the use of a saline concentration of <5% (73%, 95% CI 63–82%) compared to ≥5% (75%, 95% CI 59–89%), or according to studies reporting no use of FOB (81%, 95% CI 70–90%) compared to studies reporting the use of FOB (58%, 95% CI 38–77%), which means that FOB usage was not independently associated with a lower diagnostic yield of IS.[16] In our study, the diagnostic yield of IS was high (84%), including positive cultures at diagnosis of TB and during TB treatment with a saline concentration of 5%.

The performance of microscopy compared to culture of the IS sample (reference standard) was compared in a meta-analysis that assessed the accuracy of sputum smear examination with strategies for obtaining sputum on 1 day compared with strategies

Table 1
Concordance of sample quality between induced and spontaneous sputum

Quality of sputum sample	Percentage of concordance between IS and SS (n/N)	Percentage of discordance	
		Better quality of IS compared to SS (n)	Worse quality of IS compared to SS (n)
Consistency	74.8 (305/408)	14.2 (58)	11.0 (45)
Presence of mucus	50.2 (206/410)	26.1 (107)	23.7 (97)
Presence of blood	99.2 (378/381)	0.5 (2)	0.3 (1)
Volume of sputum	41.7 (171/410)	40.2 (165)	18.1 (74)

IS, induced sputum; SS, spontaneous sputum.

Table 2
Concordance of culture results between spontaneous and induced sputum at diagnosis and during tuberculosis treatment

	Induced sputum	
Spontaneous sputum	Positive	Negative
Positive	47	11
Negative	11	348

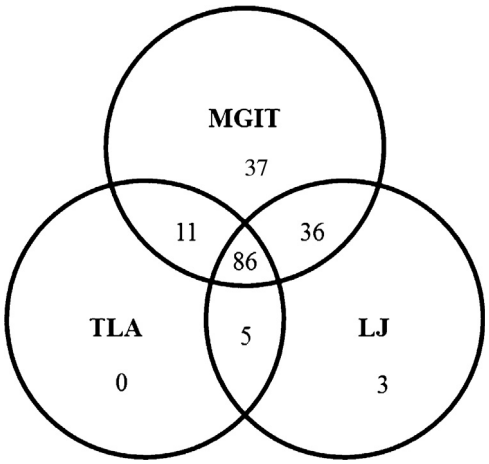

Figure 2. Agreement for positive cultures by each technique: Lowenstein–Jensen (LJ), thin-layer agar (TLA), and Mycobacteria Growth Indicator Tube (MGIT). Among 1118 cultures obtained at diagnosis and during follow-up (586 induced sputum and 532 spontaneous sputum), there were 178 positive cultures by at least one technique.

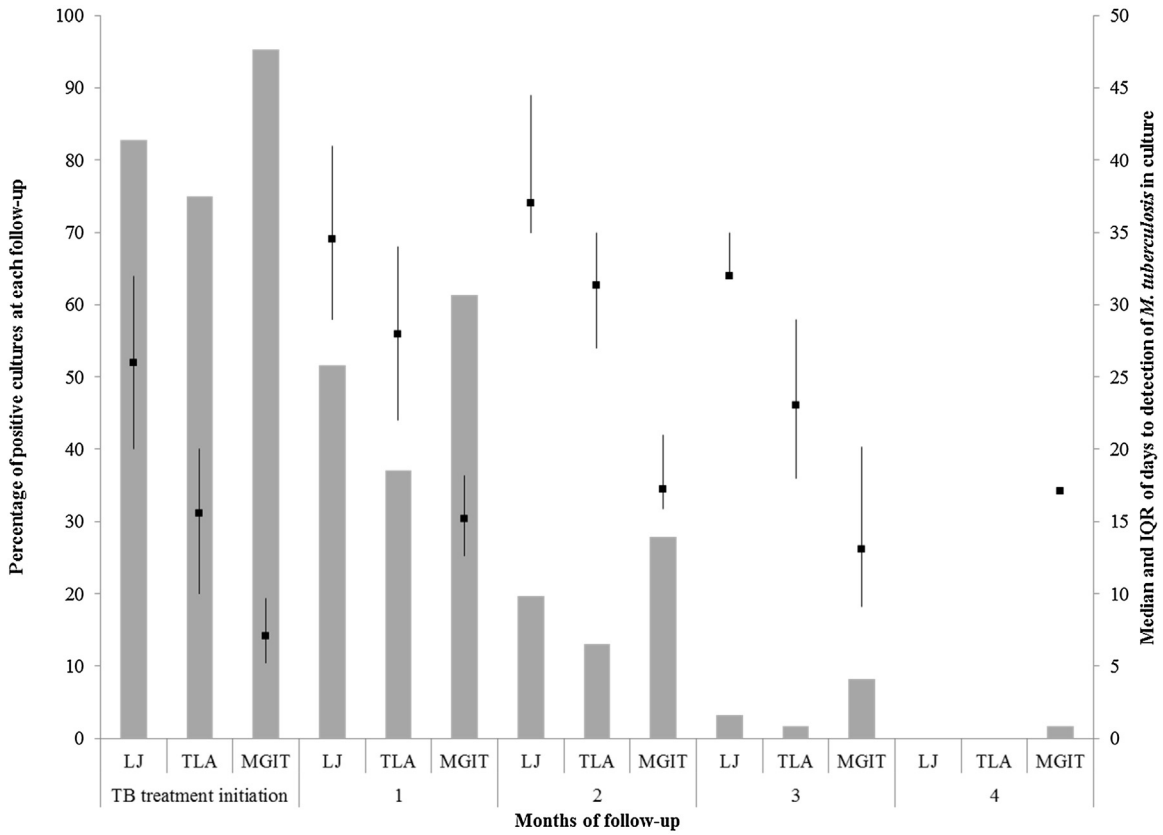

Figure 3. Time to detection of *Mycobacterium tuberculosis* in three cultures at TB treatment initiation and during follow-up. The left *y*-axis (gray bars) represents the percentage of positive cultures by each technique for the total patients at each follow-up (64 patients at treatment initiation, 62 at month 1, 61 at months 2 and 3, and 60 at month 4). The right *y*-axis represents the median (black squares) and the interquartile range (black lines) of days to detection of *M. tuberculosis* in Lowenstein–Jensen, thin-layer agar, and MGIT. The *x*-axis is the month of follow-up; only 4 months are shown because there was no positive culture after the 4th month.

for obtaining sputum over 2 days. The analysis revealed that the sensitivity of sputum smear by Ziehl–Neelsen light microscopy was 63% and 64%, respectively, and by light-emitting diode fluorescence microscopy was 69% and 73%, respectively.[27] Another systematic review of 15 studies illustrated that the sensitivity of microscopy ranges from 0.0% (95% CI 0.00–26.5%) to 77.8% (95% CI 60.80–89.90%).[15] This wide range can be explained by the heterogeneity of the studies in terms of study design (cohort and cross-sectional studies), population (children, adults, HIV-positive and HIV-negative people), age (mean range between 38 months and 90 years), and comparison with other sampling methods. In addition, authors did not relate the culture technique used in each study (solid or liquid method). As both previous studies have shown, microscopy has a low sensitivity and this demonstrates the importance of using cultures; however, it is important to use liquid media to identify *M. tuberculosis* in prisons and to use the drug susceptibility test due to the high incidence of TB in these overcrowded settings.[1,16] A rapid diagnosis in prisons will result in earlier anti-TB treatment and hence a decrease in the rate of TB transmission. The time to detection of a positive culture with MGIT was found to be shorter than with LJ or TLA, in accordance with the results of previous studies.[5–14] Furthermore, Balabanova et al. reported that the concordance between drug susceptibility testing on LJ and MGIT was 96.8% for rifampicin and 95.6% for isoniazid.[28]

Table 3
Comparison of the time to detection of *Mycobacterium tuberculosis* in three cultures by AFB sputum smear results

	Lowenstein–Jensen Median (IQR)	Thin-layer agar Median (IQR)	MGIT Median (IQR)
AFB smear-positive	27 (20–33)	13 (11–22)	7 (5–12)
AFB smear-negative	34 (28–41)	24 (20–32)	15 (11–18)

AFB, acid-fast bacillus; MGIT, Mycobacteria Growth Indicator Tube; IQR, interquartile range.

There are new molecular techniques that perform well, allowing diagnosis on the same day, with the added advantage of providing information about resistance to isoniazid and/or rifampicin. One of these techniques is the Xpert MTB/RIF assay, which has a pooled sensitivity of 88% and pooled specificity of 98%; however, sensitivity decreases to 68% when the patient has a smear-negative microscopy result. Another aspect that must be taken into account with this assay is that most of the reported studies were conducted in reference laboratories and in high TB-burden countries.[29] Cost is an advantage of the Xpert assay since, from a laboratory perspective, Xpert MTB/RIF costs $14.93/sample compared to $16.88/sample when using conventional automated liquid culture-based methods.[30] At the time of our study, this assay was not available, but it should be evaluated in further studies to diagnose TB in prisons.

This study had the following limitations. First, because all sputum samples were taken to study TB, the laboratory did not evaluate squamous cells or epithelial cells, which could have provided a quantitative evaluation of the quality of the sputum samples. Second, the IS sample and the first SS sample were taken on the same day, which could explain why there were positive cases for each technique that were negative for the other. It is important to evaluate whether this will hold true when samples are taken on separate days. Finally, another limitation could be that we used a compressor with an output of 0.25 ml/min instead of an ultrasonic nebulizer that has an output of ~1 ml/min, the value

recommended by Paggiaro et al.;[31] this output was recommended because a study reported higher success rates with the latter in terms of countable cytospins and total cell counts,[32] and due to the fact that the size of the inhaled particles affects their penetration index (airway deposition and distribution).[33] In contrast, there are two studies that have used an ultrasonic nebulizer with lower output that have shown this variable to have little influence on the outcome.[34,35] This has not been evaluated systematically, and in the worst-case scenario, the number of positive cultures obtained by IS has been underestimated.

In conclusion, our study shows that IS is safe and feasible in the prison setting, so clinicians can order this procedure when the patient is unable to provide an adequate sample or when the patient does not produce SS. IS and SS can be used for the identification of TB, while IS can be used during follow-up. The use of liquid cultures such as MGIT is very useful for the control of TB in these settings because it has the highest percentage of positivity and the shortest time to detection of *M. tuberculosis* in culture. These features allow the earlier diagnosis of pulmonary TB cases, as well as the identification of those who continue to have positive culture results during follow-up. Due to overcrowding, it is very important to identify TB cases in order to control TB transmission in prisons.

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Ethics statement: The Ethics Committee of the School of Public Health (Facultad Nacional de Salud Pública) of the Universidad de Antioquia, the Instituto Nacional Penitenciario y Carcelario (INPEC), and the director of each prison where the study was conducted approved this research. The participants were given a consent form; the form was explained and after ensuring that the participant understood the study, the consent form was signed in the presence of two witnesses (prisoners). At each follow-up, prisoners were asked for their permission for sputum induction and the collection of spontaneous expectorated sputum. Participants could withdraw consent at any stage of the study. All screened individuals, whether they entered the study or not (or withdrew during the study), received the same access to health care services and treatment when needed.

Conflict of interest: The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2015.01.004.

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