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## **Sampling strategy for bacteriological diagnosis of intrathoracic tuberculosis**

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## **Abstract**

### *Background.*

Pulmonary tuberculosis (TB) is the most frequent site of TB and the one leading its spread worldwide. Multiple specimens are commonly collected for TB diagnosis including those requiring invasive procedures. This study aimed to review the sampling strategy for the microbiological diagnosis of pulmonary TB.

### *Methods.*

A retrospective analysis of collected samples from September 1<sup>st</sup> 2014 to May 1<sup>st</sup> 2016 in the Bacteriology laboratory of Pitié-Salpêtrière Hospital (Paris, France) was performed. All the samples collected in patients aged over 18 years for the bacteriological diagnosis of pulmonary TB were included.

### *Results.*

A total of 6,267 samples were collected in 2,187 patients. One hundred and twenty-six patients (6%) had a culture confirmed pulmonary TB. Among them, multiple sputum collections were sufficient for TB diagnosis in 63.5%, gastric lavages permitted to avoid bronchoscopy in only 7.1%, and bronchoscopy was necessary in 29.4%. The culture positivity of sputa (8.6%) was higher than that of bronchial aspirations (3.1%), bronchiolo-alveolar lavages (BAL) (2.3%) or gastric lavages (4.8%) ( $p < 0.001$ ). From its 70.0% theoretical PPV value, the 46.1% selection in bronchial aspirations allocated to molecular test increased PPV up to 88.9%.

### *Conclusions.*

Based on our data, we suggest to collect sputum consistently. If smear negative a bronchoscopy should be performed and molecular diagnosis be performed on a subset of bronchial aspirations based on expertise of the bronchoscopist.

## 1. Introduction

The global threat of tuberculosis (TB) remains undiminished with the WHO estimating there were 10 million incident cases worldwide in 2018 [1]. In 2020, France is a low TB incidence country [2] but TB remains a significant public health problem, particularly in some geographic areas [3–7] and susceptible populations [4,6,8]. Pulmonary TB includes pulmonary, and mediastinal lymphadenopathy involvements [9]. It constitutes about 80% of total TB cases [1] and is the main cause of bacilli spread in the environment [10–12]; thus driving TB epidemic worldwide. The definite diagnosis of active pulmonary TB requires the culture isolation of *Mycobacterium tuberculosis* complex (M. tb) from respiratory sample [13]. Molecular tests are increasingly used in TB diagnosis, allowing a significant reduction of its delay compared to culture [14–16] and early treatment initiation. The positive predictive value (PPV) of molecular tests directly performed on a specimen is correlated to its expected culture positivity, depending on the local epidemiology of TB [17]. Significant attention must be paid in the choice of the specimen allocated to molecular tests among the multiple ones which are sampled for the diagnosis of pulmonary TB, particularly in a low TB incidence setting such as France since PPV's specimens are expected to be lower than in high TB incidence countries. Multiple sampling [18–20] are provided by a non-invasive procedure like sputum collection eventually after induction [21] or, if necessary, by an invasive procedure such as bronchoscopy or gastric lavages. Post-bronchoscopy sputum specimens have to be collected from all adults with suspected pulmonary TB who undergo bronchoscopy [21,22]. Among per-bronchoscopy sampling procedures, endobronchial ultrasound transbronchial needle aspiration (TBNA) has recently demonstrated interesting safety and sensitivity in *M. tb* isolation in hilar and mediastinal lymphadenopathy [23–29]. A reasoned use of invasive procedures requires knowing the individual profitability of their relative sample in the microbiological diagnosis of TB, compared to non-invasive, inexpensive, and rapid sampling such as sputum with or without induction. This study aimed to review the bacteriological diagnosis of pulmonary TB and to assess the contribution of invasive procedure to diagnosis, in a real life context.

## 2. Materials and methods

### 2.1. Study population

We performed a retrospective cohort study including all patients over 18 years of age who were sampled for bacteriological diagnosis of TB, hospitalized in Pitié-Salpêtrière Hospital (Paris, France), between September 1<sup>st</sup>, 2014 and May 1<sup>st</sup>, 2016.

### 2.2. Specimen

Microbiological results from sputum, gastric lavages, bronchial aspirations, bronchiolo-alveolar lavage (BAL), bronchial biopsies and TBNA were extracted from the laboratory management software GLIMS. Post-bronchoscopy sputum was defined as sputum collected during the three days following a bronchoscopy.

In the pulmonary TB diagnosis approach, multiple sputa were collected in first line. In sputum scarce or when 3 sputa were smear negative, an invasive procedure was performed *i.e.* bronchoscopy or gastric lavages. When gastric lavage was considered, its place in the local approach was always in first line, *i.e.* before bronchoscopy. All specimens were categorized as invasive except pre-bronchoscopy sputa which were not collected within the 3 days following bronchoscopy, named pre-bronchoscopy sputa.

### 2.3. Statistics

The proportions were compared with the bilateral Fisher exact test calculated using GraphPad prism software. All hypotheses were tested at alpha risk of 0.05.

### 2.4 Molecular test's PPV

The molecular test used was the DX MTB ASSAY (PCR) during the study period. According to local diagnostic strategy set up with the bronchoscopy ward, this test was preferentially done on bronchoscopy specimens. This strategy was supported by the fact that patients undergoing bronchoscopy have by definition smear negative specimens and are at high risk of TB and are evaluated by a small number of doctors that usually have a computed tomography (CT) scan to help them. As a consequence, the doctor performing the bronchoscopy was considered as the best for deciding if a PCR needed to be performed for TB diagnosis. We compared the actual PPV in our population to the theoretical PPV if the PCR had been performed on all bronchial specimens. According to the literature the sensitivity of the DX MTB ASSAY is 58% on smear negative specimens and specificity 99% [30].

The prevalence corresponding to the proportion of culture positivity, the specimens PPV were calculated by the following formula:  $PPV = \frac{Se \times Prevalence}{[Se \times Prevalence + (1 - Prevalence) \times (1 - Sp)]}$ .

### 3. Results

#### 3.1. Study population and diagnostic procedure

From September 1<sup>st</sup>, 2014 to May 1<sup>st</sup>, 2016, a total of 2,187 patients benefited from at least one microbiological sampling for pulmonary TB suspicion and were retrospectively included (Figure 1). Among invasive procedures, bronchoscopy was performed in 1,317 patients (60.2%). A total of 126 patients (5.8%) had a culture confirmed pulmonary TB. Among them, pre-bronchoscopy sputum was sufficient to diagnose their TB in 80 patients (63.5%), whereas the diagnosis required specimens collected after an invasive procedure for the remaining 46 patients (36.5%), (gastric lavage in 9/46 patients (19.5%), bronchoscopy in 37/46 patients (80.4%)) (Figure 2). Among these 37 TB patients benefiting from bronchoscopy, the majority had culture positivity from bronchial aspirations 24/37 (64.9%), followed by BAL 6/37 (16.2%), post-bronchoscopy sputum 5/37 (13.5%) and TBNA 2/37 (5.4%).

Among patients who had *M. tb* positive culture from bronchial (n=1) or TBNA (n=4) biopsies, these invasive samples were the only ones allowing the diagnosis in 0.0% and 50.0% of patients, respectively. In the 99 patients with at least one culture positive sputum, more than one sputum collection was necessary to diagnose TB in 28.3% and more than 3 in 10.1% (Table 2).

#### 3.2 Specimen's smear and culture positivity for *Mycobacterium tuberculosis*

A total of 6,267 specimens were collected and analysed. Among them, those exhibiting the highest proportion of *M. tb* culture positivity were in decreasing order, bronchial biopsies, TBNA, sputa, gastric lavages, bronchial aspirations, and finally BAL, which exhibited the lowest proportion of culture positivity. The proportion of culture positivity in sputum was higher than (Table 1) in bronchial aspirations (8.6% vs. 3.1%  $p < 0.0001$ ), BAL (8.6% vs. 2.3%  $p < 0.0001$ ) or gastric lavages (8.6% vs. 4.8%  $p = 0.0004$ ). The proportion of culture positivity in TBNA was higher than in bronchial aspirations (13.3% vs. 3.1%  $p < 0.0001$ ) and BAL (13.3% vs. 2.3%  $p < 0.0001$ ), but not compared to sputum (13.3% vs. 8.6%  $p = 0.09$ ).

Specimens exhibiting the highest proportion of smear positivity were in decreasing order, sputa, TBNA, gastric lavage, BAL, bronchial aspiration and finally bronchial biopsies (Table 1). The smear positivity was higher in sputum than in gastric lavage (5.2% vs. 2.8%  $p=0.007$ ), bronchial aspirations (5.2% vs. 0.3%  $p<0.0001$ ) and BAL (5.2% vs. 0.5%  $p<0.0001$ ). The smear positivity in TBNA was higher than in bronchial aspirations (3.3% vs. 0.3%  $p<0.0001$ ), and BAL (3.3% vs. 0.5%  $p<0.0001$ ).

### *3.3 Theoretical and actual PPV values of molecular test directly performed on smear negative bronchial aspirations*

PCR was proposed on smear negative bronchial aspirations of patients with a high suspicion of pulmonary tuberculosis as assessed by the bronchoscopist based on the analysis of the clinical file and the CT scan of the patient. This selection retained 46% of all bronchial aspirations for PCR (627/1354). Among those, the proportion of culture positivity was significantly higher than among those not retained for molecular testing (4.62% vs 1.64%,  $p=0.002$ ). Consequently, the positive predictive value (PPV) of molecular testing was high (88.9%) to be compared with a 70% theoretical PPV if molecular testing had been performed on all bronchial aspirations without the selection performed by the bronchoscopist (Table 3).

## **4. Discussion**

We present a large overview of the global interest of the different type of specimens in the bacteriological diagnosis of pulmonary TB. This study was performed in a reference hospital located in a TB hot spot region of France [3–7], permitting an overview of more than 6,000 specimens collected in a low TB incidence country. Globally, the specimen showing the main utility in pulmonary TB diagnosis is sputum in both culture and smear positivity; the multiple collection of this specimen appeared sufficient to confirm the diagnosis in two-thirds of patients. As in other studies [18,19], we showed a particular interest to collect multiple sputum to increase diagnosis accuracy. In the last third, only 20% obtained their diagnosis by gastric lavages whereas the others required specimens sampled during or after bronchoscopy. Among the different samples categorized as invasive in this study, post-bronchoscopy sputum which is the easiest to sample should be systematically realized just after or the day after the bronchoscopy and simply repeated, showed, in this study, a particular interest with a high proportion of culture and smear positivity.

These excellent diagnostic performances of sputa were obtained in our centre without having optimized the pre-analytical procedures. Some interventions have demonstrated that the sensitivity of sputa can be further increased. A particular attention should be paid to the sputum quality and quantity [21,31]. Notably, a sputum volume over 4 [32] or 5 mL [33] increases significantly smear positivity. In a recent study, Datta *et al.* showed that two simple interventions increased the diagnosis performance of microbiological testing. Pooling of sputum samples collected over a period of several hours increased the diagnosis performance of smear microscopy 1.6 times and of culture 1.7 times, compared with collection of a single time point or spot samples and the delivery of standardized instruction before the provision of sputum samples increased the performance of smear microscopy 1.4 times compared to the standard of care [34].

We suggest that gastric lavage should be avoided in practice since (i) this invasive procedure is particularly uncomfortable, (ii) it has to be repeated on several mornings and (iii) it rarely permits to avoid bronchoscopy [35] (only 20% of our TB patients). Moreover, whereas bronchoscopy allows diagnosing several different diseases, gastric lavages are limited to TB. Since the superiority of bronchoscopy compared to multiple gastric lavages is also supported by other studies [35,36], we suggest to perform bronchoscopy immediately after three smear negative sputa. In this relatively infrequent situation when bronchoscopy is necessary, we advise to collect widely all available specimens in order to benefit the most from invasive procedure utility in pulmonary TB diagnosis. Neither TBNA nor BAL collections worsen respiratory failure compared to patients receiving bronchoscopy without these samplings [24,37]. Nonetheless, contrary to BAL, TBNA procedure should be limited to patients with mediastinal or hilar lymphadenopathy visible in thoracic CT scan and accessible to endo-bronchial ultrasound-guided (EBUS) TBNA. In this last situation TBNA provides valuable support in the diagnosis of pulmonary TB [23–29], particularly in necrotic lymph nodes [29,38] and when ultrasound findings reveal clear boundaries and calcifications [28]. Above all, post-bronchoscopy sputum should be systematically collected after bronchoscopy each time BAL is performed, in view of its utility highlighted in our study and in others [39,40], to benefit to the full extent of the bronchoscopy.

Molecular tests are increasingly used in TB diagnosis, allowing a significant reduction of delay compared to culture [14–16]. Their main utility is in smear negative specimens which correspond to the most difficult to diagnose TB cases. However, the PPV of molecular testing is limited in this situation by the very small proportion of true positives, *i.e.* culture positive specimens. To help physician pre-test scores



would be useful. Such scores have mainly been assessed in extra-pulmonary TB since molecular diagnosis accuracy is particularly affected by their low bacillary load. The utility of this type of score was confirmed repeatedly in TB meningitis (TBM) [41,42], and was recently included in a consensual TBM diagnosis classification [43]. An equivalent score would also be helpful for pulmonary TB; it could be based on those validated to determine the need of early respiratory isolation before *M. tb* isolation [44,45]. In this study rather than using a score, we decided to rely on a small team of trained clinicians, the bronchoscopy team. The advantage of this organization is that this small team it is trained for difficult diagnoses and that they usually have extensive imaging. This organization reduced by approximately 50% the number of bronchial aspirations to be submitted for molecular testing and increased its PPV from 70 to 89%. On the other hand, this reduction missed 29% of culture positive bronchial aspirations (12/41) that could have been diagnosed earlier if they had been subjected to molecular testing. In conclusion, regarding molecular test's contribution to TB diagnosis, we propose that until a validated score is available, the indication of molecular testing relies on a small team having expertise and complete medical and radiological information such as the bronchoscopy team in our hospital. Our study was limited by the lack of clinical and radiological detailed information, which did not allow us to grade the pre-test probability of pulmonary TB. Notably, the diagnosis accuracy of biopsies was hard to assess since, contrary to BAL or bronchial aspirations, which were systematically performed during bronchoscopy, they were sampled only in specific patients with radiologic or macroscopic arguments for accessible lymphadenopathy or bronchial TB involvements. This real life study is naturally limited by the fact that diagnosis procedure was not prospectively controlled even if included patient's diagnosis followed the same approach. An explanation of the sputum highest accuracy in TB diagnosis could be that sputum was used for the easiest to diagnose cases that harbour the highest bacillary loads. Nonetheless, we confirmed the importance of post-bronchoscopy sputa whereas in our study those patients had pauci-bacillary TB.

## 5. Conclusion

This study confirms the higher accuracy of sputum in the microbiological diagnosis of pulmonary TB compared to invasive specimens in our context of low TB incidence. Based on our data, we suggest collecting sputum consistently, and then if both smear and molecular test remain negative, bronchoscopy should be proposed not forgetting post-bronchoscopy sputa. Molecular testing can be performed following indication by the bronchoscopist.

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## 234 **Disclosure of interest**

235 The authors declare that they have no competing interest.

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## 237 **References**

- 238 [1] World Health Organization. Global tuberculosis report 2019. S.I.: 2019.  
239 [2] Story A, Antoine D. // TUBERCULOSIS AND HEALTH INEQUALITIES n.d.:39.  
240 [3] Pierre-Audigier C, Talla C, Alame-Emane A-K, Audigier B, Grall N, Ruimy R, et al. Tuberculosis  
241 trends in a hot-spot region in Paris, France. *Int J Tuberc Lung Dis Off J Int Union Tuberc Lung Dis*  
242 2020;24:428–35. <https://doi.org/10.5588/ijtld.19.0305>.  
243 [4] Nienhaus A, Garipey P-K, Trouve C, Lhaumet C, Toureau J, Peters C. Tuberculosis screening  
244 at the Sainte-Anne Hospital in Paris – results of first and second IGRA. *J Occup Med Toxicol* 2014;9:1–  
245 5. <https://doi.org/10.1186/1745-6673-9-24>.  
246 [5] Lanoix J-P, Gaudry S, Flicoteaux R, Ruimy R, Wolff M. Tuberculosis in the intensive care unit:  
247 a descriptive analysis in a low-burden country. *Int J Tuberc Lung Dis Off J Int Union Tuberc Lung Dis*  
248 2014;18:581–7. <https://doi.org/10.5588/ijtld.13.0901>.  
249 [6] Bernard C, Sougakoff W, Fournier A, Larnaudie S, Antoun F, Robert J, et al. Impact of a 14-  
250 year screening programme on tuberculosis transmission among the homeless in Paris. *Int J Tuberc*  
251 *Lung Dis Off J Int Union Tuberc Lung Dis* 2012;16:649–55. <https://doi.org/10.5588/ijtld.11.0241>.  
252 [7] Valade S, Raskine L, Aout M, Malissin I, Brun P, Deye N, et al. Tuberculosis in the intensive  
253 care unit: A retrospective descriptive cohort study with determination of a predictive fatality score. *Can*  
254 *J Infect Dis Med Microbiol* 2012;23:173–8.  
255 [8] Gehanno J-F, Abiteboul D, Rollin L. Incidence of tuberculosis among nurses and healthcare  
256 assistants in France. *Occup Med Oxf Engl* 2017;67:58–60. <https://doi.org/10.1093/occmed/kqw138>.  
257 [9] Kang W, Yu J, Du J, Yang S, Chen H, Liu J, et al. The epidemiology of extrapulmonary  
258 tuberculosis in China: A large-scale multi-center observational study. *PloS One* 2020;15:e0237753.  
259 <https://doi.org/10.1371/journal.pone.0237753>.  
260 [10] Urbanowski ME, Ordonez AA, Ruiz-Bedoya CA, Jain SK, Bishai WR. Cavitory tuberculosis: the  
261 gateway of disease transmission. *Lancet Infect Dis* 2020;20:e117–28. [https://doi.org/10.1016/S1473-3099\(20\)30148-1](https://doi.org/10.1016/S1473-3099(20)30148-1).  
262 [11] Martinez L, Verma R, Croda J, Horsburgh CR, Walter KS, Degner N, et al. Detection, survival  
263 and infectious potential of Mycobacterium tuberculosis in the environment: a review of the evidence and  
264 epidemiological implications. *Eur Respir J* 2019;53. <https://doi.org/10.1183/13993003.02302-2018>.  
265 [12] Donald PR, Diacon AH, Lange C, Demers A-M, von Groote-Bidlingmaier F, Nardell E. Droplets,  
266 dust and guinea pigs: an historical review of tuberculosis transmission research, 1878-1940. *Int J Tuberc*  
267 *Lung Dis Off J Int Union Tuberc Lung Dis* 2018;22:972–82. <https://doi.org/10.5588/ijtld.18.0173>.  
268 [13] Schoch OD, Rieder P, Tueller C, Altpeter E, Zellweger J-P, Rieder HL, et al. Diagnostic Yield of  
269 Sputum, Induced Sputum, and Bronchoscopy after Radiologic Tuberculosis Screening. *Am J Respir Crit*  
270 *Care Med* 2007;175:80–6. <https://doi.org/10.1164/rccm.200608-1092OC>.  
271 [14] Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid Molecular  
272 Detection of Tuberculosis and Rifampin Resistance. *N Engl J Med* 2010;363:1005–15.  
273 <https://doi.org/10.1056/NEJMoa0907847>.  
274 [15] Seki M, Kim C-K, Hayakawa S, Mitarai S. Recent advances in tuberculosis diagnostics in  
275 resource-limited settings. *Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol* 2018;37:1405–  
276 10. <https://doi.org/10.1007/s10096-018-3258-y>.  
277

- [16] McNerney R, Zumla A. Impact of the Xpert MTB/RIF diagnostic test for tuberculosis in countries with a high burden of disease. *Curr Opin Pulm Med* 2015;21:304–8. <https://doi.org/10.1097/MCP.000000000000161>.
- [17] Pham T-T, Schrenzel J, Janssens J-P, Emonet S. Performances and usefulness of Xpert MTB/RIF assay in low-incidence settings: not that bad? *Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol* 2020. <https://doi.org/10.1007/s10096-020-03887-8>.
- [18] Nelson SM, Deike MA, Cartwright CP. Value of examining multiple sputum specimens in the diagnosis of pulmonary tuberculosis. *J Clin Microbiol* 1998;36:467–9.
- [19] Mase SR, Ramsay A, Ng V, Henry M, Hopewell PC, Cunningham J, et al. Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review. *Int J Tuberc Lung Dis Off J Int Union Tuberc Lung Dis* 2007;11:485–95.
- [20] Yoo H, Song J-U, Koh W-J, Jeon K, Um S-W, Suh GY, et al. Additional role of second washing specimen obtained during single bronchoscopy session in diagnosis of pulmonary tuberculosis. *BMC Infect Dis* 2013;13:404. <https://doi.org/10.1186/1471-2334-13-404>.
- [21] Lewinsohn DM, Leonard MK, LoBue PA, Cohn DL, Daley CL, Desmond E, et al. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children. *Clin Infect Dis* 2017;64:e1–33. <https://doi.org/10.1093/cid/ciw694>.
- [22] Forbes BA, Hall GS, Miller MB, Novak SM, Rowlinson M-C, Salfinger M, et al. Practical Guidance for Clinical Microbiology Laboratories: Mycobacteria. *Clin Microbiol Rev* 2018;31. <https://doi.org/10.1128/CMR.00038-17>.
- [23] Geake J, Hammerschlag G, Nguyen P, Wallbridge P, Jenkin GA, Korman TM, et al. Utility of EBUS-TBNA for diagnosis of mediastinal tuberculous lymphadenitis: a multicentre Australian experience. *J Thorac Dis* 2015;7:439–48. <https://doi.org/10.3978/j.issn.2072-1439.2015.01.33>.
- [24] Navani N, Molyneaux PL, Breen RA, Connell DW, Jepson A, Nankivell M, et al. Utility of endobronchial ultrasound-guided transbronchial needle aspiration in patients with tuberculous intrathoracic lymphadenopathy: a multicentre study. *Thorax* 2011;66:889–93. <https://doi.org/10.1136/thoraxjnl-2011-200063>.
- [25] Kanda H, Uchimura K, Hara S, Sakakibara H, Morimoto T, Shigemi S, et al. [A Retrospective Study of Endobronchial Ultrasound-guided Transbronchial Needle Aspiration (EBUS-TBNA) Performed on Tuberculous Lymphadenitis Cases]. *J UOEH* 2020;42:223–7. <https://doi.org/10.7888/juoeh.42.223>.
- [26] Ko R-E, Jeong B-H, Chon HR, Huh HJ, Han J, Lee H, et al. Clinical usefulness of routine AFB culture and MTB PCR of EBUS-TBNA needle rinse fluid. *Respirology* 2019;24:667–74. <https://doi.org/10.1111/resp.13488>.
- [27] Sun J, Teng J, Yang H, Li Z, Zhang J, Zhao H, et al. Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration in Diagnosing Intrathoracic Tuberculosis. *Ann Thorac Surg* 2013;96:2021–7. <https://doi.org/10.1016/j.athoracsur.2013.07.005>.
- [28] Yang J, Du Z. Comparison of clinical and pathological features of lymph node tuberculosis and histiocytic necrotizing lymphadenitis. *J Infect Dev Ctries* 2019;13:706–13. <https://doi.org/10.3855/jidc.10860>.
- [29] Lin C-K, Keng L-T, Lim C-K, Lin Y-T, Lin S-Y, Chen L-Y, et al. Diagnosis of mediastinal tuberculous lymphadenitis using endobronchial ultrasound-guided transbronchial needle aspiration with rinse fluid polymerase chain reaction. *J Formos Med Assoc* 2020;119:509–15. <https://doi.org/10.1016/j.jfma.2019.07.014>.
- [30] BIORAD. DX MTB ASSAY test procedure. Direct detection of Mycobacterium tuberculosis complex by real-time PCR. 2013. <https://doi.org/10.4000/books.septentrion.19822>.
- [31] Ho J, Marks GB, Fox GJ. The impact of sputum quality on tuberculosis diagnosis: a systematic review. *Int J Tuberc Lung Dis Off J Int Union Tuberc Lung Dis* 2015;19:537–44. <https://doi.org/10.5588/ijtld.14.0798>.
- [32] Yoon SH, Lee NK, Yim JJ. Impact of sputum gross appearance and volume on smear positivity of pulmonary tuberculosis: a prospective cohort study. *BMC Infect Dis* 2012;12:172. <https://doi.org/10.1186/1471-2334-12-172>.
- [33] Fisher M, Dolby T, Surtie S, Omar G, Hapeela N, Basu D, et al. Improved method for collection of sputum for tuberculosis testing to ensure adequate sample volumes for molecular diagnostic testing. *J Microbiol Methods* 2017;135:35–40. <https://doi.org/10.1016/j.mimet.2017.01.011>.
- [34] Datta S, Shah L, Gilman RH, Evans CA. Comparison of sputum collection methods for tuberculosis diagnosis: a systematic review and pairwise and network meta-analysis. *Lancet Glob Health* 2017;5:e760–71. [https://doi.org/10.1016/S2214-109X\(17\)30201-2](https://doi.org/10.1016/S2214-109X(17)30201-2).

- [35] Dickson SJ, Brent A, Davidson RN, Wall R. Comparison of bronchoscopy and gastric washings in the investigation of smear-negative pulmonary tuberculosis. *Clin Infect Dis Off Publ Infect Dis Soc Am* 2003;37:1649–53. <https://doi.org/10.1086/379716>.
- [36] Rizvi N, Rao NA, Hussain M. Yield of gastric lavage and bronchial wash in pulmonary tuberculosis. *Int J Tuberc Lung Dis Off J Int Union Tuberc Lung Dis* 2000;4:147–51.
- [37] Cracco C, Fartoukh M, Prodanovic H, Azoulay E, Chenivresse C, Lorut C, et al. Safety of performing fiberoptic bronchoscopy in critically ill hypoxemic patients with acute respiratory failure. *Intensive Care Med* 2013;39:45–52. <https://doi.org/10.1007/s00134-012-2687-9>.
- [38] Rodriguez-Takeuchi SY, Renjifo ME, Medina FJ. Extrapulmonary Tuberculosis: Pathophysiology and Imaging Findings. *RadioGraphics* 2019;39:2023–37. <https://doi.org/10.1148/rg.2019190109>.
- [39] George PM, Mehta M, Dhariwal J, Singanayagam A, Raphael CE, Salmasi M, et al. Post-bronchoscopy sputum: improving the diagnostic yield in smear negative pulmonary TB. *Respir Med* 2011;105:1726–31. <https://doi.org/10.1016/j.rmed.2011.07.014>.
- [40] Malekmohammad M, Marjani M, Tabarsi P, Baghaei P, Sadr Z, Naghan PA, et al. Diagnostic yield of post-bronchoscopy sputum smear in pulmonary tuberculosis. *Scand J Infect Dis* 2012;44:369–73. <https://doi.org/10.3109/00365548.2011.643820>.
- [41] Patel VB, Theron G, Lenders L, Matinyena B, Connolly C, Singh R, et al. Diagnostic Accuracy of Quantitative PCR (Xpert MTB/RIF) for Tuberculous Meningitis in a High Burden Setting: A Prospective Study. *PLOS Med* 2013;10:e1001536. <https://doi.org/10.1371/journal.pmed.1001536>.
- [42] Anselmo LMP, Feliciano C, Mauad F, do Nascimento MP, Pocente RC, Silva JM, et al. A predictive score followed by nucleic acid amplification for adult tuberculous meningitis diagnosis in Southern Brazil. *J Neurol Sci* 2017;379:253–8. <https://doi.org/10.1016/j.jns.2017.06.016>.
- [43] Marais BJ, Heemskerk AD, Marais SS, van Crevel R, Rohlwink U, Caws M, et al. Standardized Methods for Enhanced Quality and Comparability of Tuberculous Meningitis Studies. *Clin Infect Dis Off Publ Infect Dis Soc Am* 2017;64:501–9. <https://doi.org/10.1093/cid/ciw757>.
- [44] Delory T, Ferrand H, Grall N, Casalino E, Lafarge M, Melot B, et al. Score for pulmonary tuberculosis in patients with clinical presumption of tuberculosis in a low-prevalence area. *Int J Tuberc Lung Dis Off J Int Union Tuberc Lung Dis* 2017;21:1272–9. <https://doi.org/10.5588/ijtld.17.0353>.
- [45] Pinto LM, Pai M, Dheda K, Schwartzman K, Menzies D, Steingart KR. Scoring systems using chest radiographic features for the diagnosis of pulmonary tuberculosis in adults: a systematic review. *Eur Respir J* 2013;42:480–94. <https://doi.org/10.1183/09031936.00107412>.

Figures

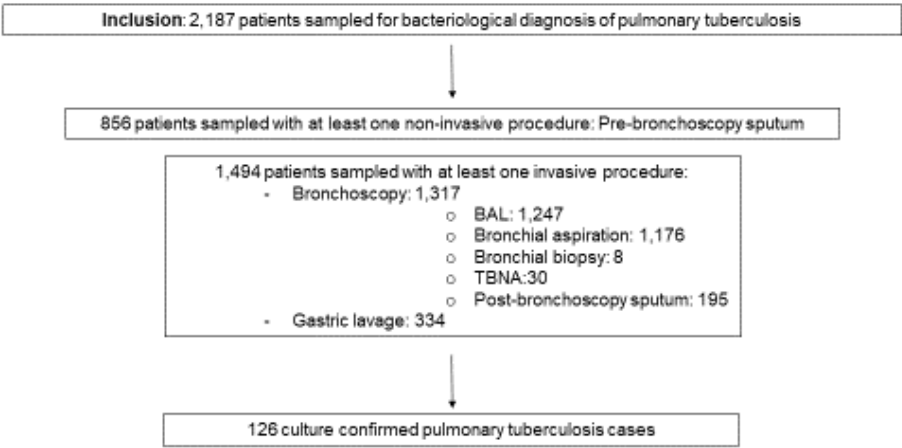
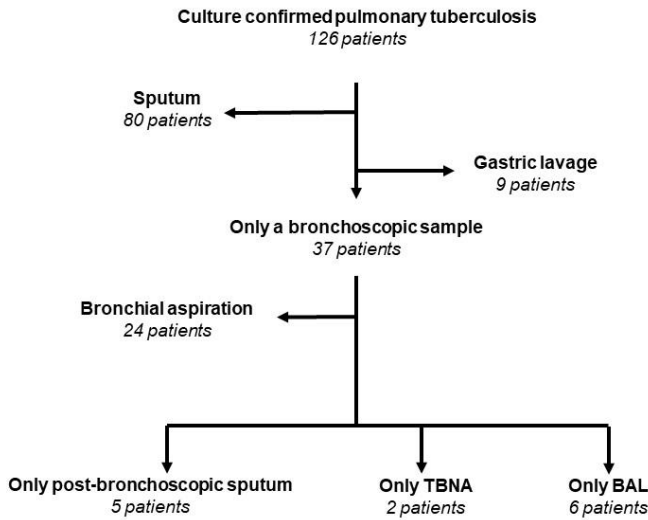


Figure 1: Flow chart of the study design



BAL: Bronchiolo-alveolar lavage

TBNA: Transbronchial needle aspiration

Figure 2: Distribution of *M. tuberculosis* complex positive culture specimens leading to 126 pulmonary tuberculosis diagnoses

## Tables

**Table 1:** Bacteriological results of the 6,267 specimens collected from September 1<sup>st</sup>, 2014 to May 1<sup>st</sup>, 2016 in patients with suspicion of pulmonary TB.

Type of specimens	n	<i>M. tuberculosis</i> culture-positive samples N (%) <sup>*</sup>	Smear-positive samples N (%) <sup>*</sup>
All sputum	2,591	222 (8.6%)	136 (5.2%)
Pre bronchoscopy sputum	2,018	169 (8.4%)	104 (5.1%)
Post bronchoscopy sputum	573	53 (9.2%)	32 (5.6%)
Gastric lavage	848	41 (4.8%)	24 (2.8%)
Bronchial aspiration	1,358	42 (3.1%)	4 (0.3%)
BAL	1,433	33 (2.3%)	7 (0.5%)
TBNA	30	4 (13.3%)	1 (3.3%)
Bronchial biopsy	7	1 (14.3%)	0 (0.0%)
<b>Total</b>	<b>6,267</b>	<b>343</b>	<b>172</b>

<sup>\*</sup> percentage among the type of sample

BAL: Bronchiolo-alveolar lavage

TBNA: Transbronchial needle aspiration

**Table 2:** Individual sputum contribution in the microbiological diagnosis of tuberculosis

Sputum	Patients with at least one culture positive sputum	The first is sufficient	The second is necessary	The third is necessary	More than 3 are necessary
All	99	71 (71.7%)	12 (12.1%)	6 (6.1%)	10 (10.1%)
Pre-bronchoscopy	81	61 (75.3%)	6 (7.4%)	5 (6.2%)	9 (11.1%)
Post-bronchoscopy	18	10 (55.6%)	6 (33.1%)	1 (5.6%)	1 (5.6%)

412 Table 3: Proportion of culture positivity theoretical and actual PPV of molecular tests directly performed  
 413 on smear negative bronchial aspirations

Smear negative bronchial aspirations	
Specimens: n	1,354
Culture positivity: n (%)	38 (2.8%)
Expected PPV*: %	70.0%
PCR performed: n (%)	624 (46.1%)
Observed PPV: %	88.9%

414 PPV: Positive predictive value

415 \* According to [30]

416