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Bleach-concentrated direct sputum smear microscopy procedure for diagnosis of pulmonary tuberculosis in poverty areas

Microscopia direta de amostra de escarro concentrado em água sanitária para diagnóstico de tuberculose pulmonar

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ABSTRACT

Introduction: Pulmonary tuberculosis caused by *Mycobacterium tuberculosis* is a serious public health problem affecting millions of people worldwide. The development of easy and low-cost diagnostic methods is crucial for disease control in rural remote and poverty areas and among vulnerable groups. **Objective:** To evaluate the accuracy of laboratory methods for the diagnosis of Pulmonary tuberculosis. **Material and methods:** Sputum samples from patients with clinical signs and symptoms were analyzed by microscopy after chemical treatment and spontaneous sedimentation and compared with methods employed routinely: direct sputum smear microscopy, culture, and GeneXpert®MTB/RIF. **Results:** From the samples analyzed, 16% were positive by microscopy in the processed samples, 18% by both culture and Xpert®MTB/RIF, while 13% in the direct microscopy. The processed samples showed a 31% increase in positivity (57 samples) compared to conventional direct microscopy. In the analysis of the accuracy of the evaluated methods, all the results were statistically significant proving that they were not randomly positive or negative and confirming that there is a tendency for these results. **Conclusion:** Chemical treatment and spontaneous sedimentation of the sputum samples procedure represent an effective diagnostic tool in situations where more advanced technologies are not feasible. Besides the higher accuracy and greater detection of positive cases regarding the direct smear, the procedure strengthens biosafety by decreasing the risks of aerial contamination by *Mycobacterium tuberculosis* for laboratory professionals.

Key words: *Mycobacterium tuberculosis*; diagnosis tuberculosis; processed sputum; bleach microscopy method.

RESUMO

Introdução: *A tuberculose pulmonar causada por* Mycobacterium tuberculosis *é um grave problema de saúde pública que afeta mundialmente milhões de indivíduos. O desenvolvimento de métodos de diagnóstico fáceis e de baixo custo é essencial para o controle da doença nas áreas rurais remotas e de pobreza e entre os grupos vulneráveis.* ***Objetivo:*** *Avaliar a acurácia dos métodos laboratoriais para o diagnóstico de tuberculose pulmonar.* ***Material e métodos:*** *As amostras de escarro de pacientes com sinais e sintomas clínicos foram analisadas por microscopia após tratamento químico e sedimentação espontânea e comparadas com métodos empregados rotineiramente: baciloscopia direta do escarro, cultura e GeneXpert® MTB/RIF.* ***Resultados:*** *Das amostras analisadas, 16% foram positivas por microscopia nas amostras processadas; 18%, por cultura e Xpert® MTB/RIF; e 13%, por microscopia direta. As amostras processadas apresentaram um aumento de 31% de positividade (57 amostras) em relação à microscopia direta convencional. Na análise dos métodos avaliados, todos os resultados foram estatisticamente significativos, comprovando que não eram positivos ou negativos aleatoriamente e confirmando que há uma tendência para esses resultados.* ***Conclusão:*** *O tratamento químico e a sedimentação espontânea das amostras de escarro representam uma ferramenta diagnóstica*

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eficaz nas situações em que tecnologias mais avançadas não são viáveis. Além da maior precisão e maior detecção de casos positivos em relação ao esfregaço direto, o procedimento reforça a biossegurança, diminuindo os riscos de contaminação aérea por Mycobacterium tuberculosis *para profissionais de laboratório.*

Unitermos: Mycobacterium tuberculosis*; diagnóstico de tuberculose; escarro processado; método de microscopia com água sanitária.*

RESUMEN

Introducción: *La tuberculosis pulmonar causada por* Mycobacterium tuberculosis *es un grave problema de salud pública que afecta millones de individuos en el mundo. El desarrollo de métodos de diagnóstico fáciles y de bajo costo es esencial para el control de la enfermedad en zonas rurales remotas y pobres y entre los grupos vulnerables.* ***Objetivo:*** *Evaluar la exactitud de métodos de laboratorio para el diagnóstico de tuberculosis pulmonar.* ***Material y métodos:*** *Las muestras del esputo de pacientes con signos y síntomas clínicos fueron analizadas por microscopía luego de tratamiento químico y sedimentación espontánea y comparados con métodos empleados ordinariamente: baciloscopía directa de esputo, cultivo y GeneXpert® MTB/RIF.* ***Resultados:*** *Entre las muestras analizadas, 16% fueron positivas por microscopía en las muestras procesadas; 18% por cultivo y Xpert® MTB/RIF; y 13% por microscopía directa. Las muestras procesadas presentaran un aumento de 31% de positividad (57 muestras) con respecto a la microscopía directa convencional. En el análisis de los métodos, todos los resultados fueron estadísticamente significativos, comprobando que no eran aleatoriamente positivos o negativos y confirmando que hay una tendencia para esos resultados.* ***Conclusión:*** *El tratamiento químico y la sedimentación espontánea de las muestras de esputo representan una herramienta diagnóstica eficaz en las situaciones en las cuales tecnologías más avanzadas no son viables. Además de la mayor precisión y mayor detección de casos positivos de lo que hace el frotis directo, el procedimiento fortalece la bioseguridad, disminuyendo los riesgos de contaminación del aire por* Mycobacterium tuberculosis *para el personal de laboratorio.*

Palabras clave: Mycobacterium tuberculosis*; diagnóstico de tuberculosis; esputo procesado; método de microscopía con hipoclorito de sodio.*

INTRODUCTION

Pulmonary tuberculosis (PTB) caused by *Mycobacterium tuberculosis* (*Mtb*) has been affecting humans for millennia and still affects millions of people annually and is considered one of the top 10 causes of death worldwide[1, 2].

The cases occur more frequently in urban agglomerations with low socioeconomic level and poor sanitary conditions. Investments for higher efficient diagnosis and treatment have somewhat contributed to restraining the disease, nevertheless, it remains a serious public health problem worldwide. In developed countries, the morbidity and mortality rates from PTB are declining. However, in regions where the prevalence of human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/Aids) is high the number of new cases and deaths is increasing[2].

The disease can be diagnosed by clinical history, chest X-ray, chest computed tomography (CT), bronchoscopy, tuberculin skin test or Mantoux reaction, however, the conclusive diagnosis requires the isolation and identification of the *Mtb* by laboratory tests such as direct smear microscopy, culture, and molecular biology techniques[1, 2]. The culture, by its high sensitivity, has been considered the gold standard by studies to compare the accuracy of other methods for PTB diagnosis. According to the laboratory standards, its detection threshold can reach 10 bacilli/ml of sputum, but it has the disadvantage of being a complex, laborious and time-consuming methodology, taking up to 8-12 weeks, before a negative *Mtb* growth result is reported, and requiring complex laboratory facilities and containment level 3 (BSL3) conditions[2, 3].

The new diagnostic methods using automated platforms, such as the GeneXpert® MTB/RIF system have high sensitivity and specificity to detect both the *Mtb* and drugs resistance in sputum samples and provide the results quickly in up to 2 hours[4]. The sensitivity of the test is around 68% to 72% in smear-negative and 98% among smear-positive *Mtb* individuals[4, 5].

The direct sputum smear microscopy (DSSM), for simplicity, rapidity, and low cost, is the most widely used method for

researching the *Mtb* among PTB clinically suspect patients, especially in places with restricted access to other diagnostic methods. However, many active PTB patients remain negative, mainly the paucibacillary cases, as children and co-infected with HIV/Aids patients. The detection threshold of DSSM is around 5,000 to 10,000 bacilli/ml of sputum. According to the quality and number of samples per patient, besides the technician skills, the sensitivity of DSSM can reach up to 90%[2].

Several studies have been carried out aiming for an increase in DSSM sensitivity and their results have been varied and the developed protocols have not been widely implemented in laboratory routine practices for PTB diagnosis[6-9]. Therefore, the development of easier and low-cost methodologies for use in limited resource places is mandatory.

This study evaluated the accuracy of a modified DSSM, with previous chemical treatment of the sputum samples with bleach [sodium hypochlorite (NaClO)] and spontaneous sedimentation, compared to routine methods (culture, Xpert® MTB/RIF, and conventional DSSM) used by public health laboratory services for PTB diagnosis.

MATERIAL AND METHODS

Samples

A convenience sample of specimens of sputum was collected from individuals with signs and symptoms of PTB from health units in Recife, Pernambuco, Brazil. An aliquot of each sample was immediately used for conventional direct sputum smear microscopy, for culture inoculation, and for molecular analyses by the Xpert® MTB/RIF (Cepheid Inc Sunnyvale, CA, USA) system and the remaining portion of sputum was used for the modified DSSM method, with chemical treatment with commercial bleach, as described below.

Direct sputum smear microscopy by conventional the method (cDSSM)

The cDSSM was performed as previously described[10]. Briefly, in a biological safety cabinet (Class II-B2), the sputum samples were macroscopically observed, and the thicker mucous portions were picked with bamboo applicator sticks and thoroughly smeared on glass microscope slides. The smears were air-dried at room temperature, heat-fixed using the flame of a spirit lamp, and stained using the standard Ziehl-Neelsen method, using Laborclin products (Pinhais, Paraná, Brazil).

The readings were carried out on a bright-field microscope with a 100× oil objective lens, and the results were recorded based on the standard scoring scheme for acid-fast bacilli (AFB) identification as follows: negative (no AFB observed in 100 fields); 1-9 AFB (1-9 AFB in 100 fields); 1+ (10-99 AFB in 100 fields); 2+ (1-10 AFB per field, after checking 50 fields); 3+ (more than 10 AFB per field after checking 20 fields)[10].

Culture of the sputum samples

The cultures were performed on the Ogawa-Kudoh medium (Laborclin – Pinhais, Paraná, Brazil), according to the usual procedure[10]. For each sample, a swab was impregnated with the most purulent portion of the sputum and introduced into a sterile tube containing 3 ml of 4% NaOH solution for two minutes for decontamination and then spread out over the surface of the culture medium. The cultures were incubated at 37ºC and observed after 48 hours to verify bacterial growth, which at this stage is indicative of contamination. The cultures were observed weekly for up to 60 days to follow the development of colonies' characteristics of *Mtb*, such as time of growth and morphological features. The *Mtb* identification was confirmed by the properties of alcohol-acid resistance, detection of cord factor, biochemical reactions of niacin, catalase, nitrate reductase, β-nitrobenzoic acid (PNB) and thiophene-2-carboxylic acid hydrazide (TCH) and antimicrobial susceptibly testing.

GeneXpert® MTB/RIF system analysis

For the GeneXpert® MTB/RIF (Cepheid Inc Sunnyvale, CA, USA) testing each sputum sample was initially treated with a reagent containing NaOH and isopropanol. The sample was then transferred manually to a single-use cartridge, pre-filled with the reaction mixture. The cartridge is inserted into the equipment which automatically runs the steps of deoxyribonucleic acid (DNA) extraction, amplification, and detection. The results are automatically generated on the screen as negative or positive *Mtb* (with a semi-quantitative estimate of the concentration reported as low to medium or high and sensitive or resistant to rifampicin)[4, 5].

Bleach-concentrated direct sputum smear microscopy (BC-DSSM)

Bleach-concentrated direct sputum smear microscopy was carried out following the procedure previously described[11]. In brief, after the conventional sputum smear had been prepared, the remnant sputum sample was mixed with a solution of 2%-2.5% sodium hypochlorite commercial bleach (Brilux, Paulista,

Pernambuco, Brazil) in a volume equal to twice the volume in the container and gently homogenized to avoid spilling; the mixture was then left for approximately 10 minutes for inactivation of the *Mtb* or others contaminating bacteria. The mixture was then poured directly into a 12 ml screw cap conical tube, mixed thoroughly to improve the chemical digestion of the organic material, and maintained at room temperature overnight (12 to 18 hours) for spontaneous sedimentation. Next, the supernatant was discarded. The remaining deposit was vigorously mixed, the lid was discarded, and the open tube was inverted in the center of a glass slide and left until its contents were completely drained onto the slide surface. The samples were then smeared over the slide using the mouth of the tube. The smears were air-dried at room temperature and stained and recorded as described[10, 11].

Statistical analysis

The results were introduced into a database built in the SPSS 20.0 for Windows and analyzed by Open Epi14 and SPSS programs to calculate the data accuracy [sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and precision]. The chi-square test was used to assess the dependence of the variables. The p-value < 0.05 was considered statistically significant[12].

ETHICAL ASPECTS

The research project was approved by the Ethics Committee of the Instituto Aggeu Magalhães (CEP-IAM) – Fiocruz/PE (CAAE 57175616.9.0000.5190) with the consent of the Health Units of the Municipal Health Secretariat of Recife, Pernambuco, Brazil.

RESULTS

Sputum samples were analyzed by GeneXpert® MTB/RIF (n = 1,346), culture (n = 1,332), conventional direct smear and processed sputum smear (n = 1,348). The difference in the number of samples was due to the exclusion of some contaminated cultures for calculating accuracy with culture as the gold standard. The accuracy of the four methodologies was analyzed statistically. The results are shown in **Tables 1**, **2**, and **3**. There was a growth of *Mtb* (positive cultures) in 18% (235/1,332) of the sputum samples; 13% of the samples (171/1,332) were positive for AFB by direct sputum smear; 16% (213/1,332) by processed sputum smear, and 18% (234/1,332) by GeneXpert® MTB/RIF (Table 1).

Comparing the performance of direct, bleach-concentrated sputum smear, and GeneXpert® MTB/RIF with the culture it was observed that out of the 235 culture-positive samples, 73% (171/235) were positive for AFB and 27% (64/235) were negative (false negative) by direct sputum smear microscopy (Table 1). From the 1,332 samples examined the direct sputum smear positivity was 13%. Regarding the BC-DSSM, 81% (191/235) were positive for AFB and 19% (44/235) were negative (false negative). On the other hand, 22 negative-culture sputum samples were positive by BC-DSSM summing up 213 positive samples. Therefore, 16% from the 1,332 sputum processed samples were positive (Table 1).

Regarding the divergent results, cultures were negative in three from 12 paucibacillary samples (1-9 AFB microscopy/100 fields); 12 from 79 positive 1+ samples and seven from 73 positive 2+ samples by BC-DSSM. There was a full agreement between the culture gold-standard method and BC-DSSM in 71 positive 3+

TABLE 1 – Comparisons of the results obtained by direct sputum smear microscopy by conventional method, culture, GeneXpert® MTB/RIF system analysis, and bleach-concentrated direct sputum smear microscopy

cDSSM	Culture		
	Positive	Negative	Total
Positive	171	10	181
Negative	64	1,087	1,151
Total	235	1,097	1,332
BC-DSSM	**Culture**		
	Positive	**Negative**	**Total**
Positive	213	22	235
Negative	22	1,075	1,097
Total	235	1,097	1,332
Gene Xpert	**Culture**		
	Positive	**Negative**	**Total**
Positive	212	32	244
Negative	22	1,066	1,088
Total	234	1,097	1,331
cDSSM	**Gene Xpert**		
	Positive	**Negative**	**Total**
Positive	176	9	185
Negative	79	1,082	1,161
Total	255	1,091	1,346
BC-DSSM	**Gene Xpert**		
	Positive	**Negative**	**Total**
Positive	223	32	255
Negative	19	1,072	1,091
Total	242	1,104	1,346
BC-DSSM	**cDSSM**		
	Positive	**Negative**	**Total**
Positive	186	57	243
Negative	0	1,105	1,105
Total	186	1,162	1,348

cDSSM: conventional direct sputum smear microscopy; BC-DSSM: bleach-concentrated processing direct sputum smear microscopy by; GeneXpert: rapid molecular assay.

TABLE 2 – Association of the AFB counts in bleach-concentrated direct sputum smear microscopy with the positive or negative cultures

BC-DSSM	Culture		
	Positive	Negative	Total
1-9 AFB	9	3	12
1+	67	12	79
2+	66	7	73
3+	71	0	71
Negative	22	1,075	1,097
Total	235	1,096	1,332

cDSSM: direct sputum smear microscopy by conventional method; AFB: acid-fast bacilli; 1-9 AFB: 1-9 AFB in 100 fields; 1+: 10-99 AFB in 100 fields; 2+: 1-10 AFB in 50 fields; 3+: > 10 AFB in 20 fields.

TABLE 3 – Evaluation of the accuracy of four laboratory methods for the diagnosis of pulmonary tuberculosis

Comparaisons	Sensitivity	Specificity	PPV	NPV	Precision	Kappa
cDSSM × Culture	73%	99%	95%	94%	94%	79%
CI	67-78	98-99	90-97	93-96	93.1-96	$p < 0.001$
BC-DSSM × Culture	91%	98%	91%	98%	97%	54%
CI	86-94	97-99	86-94	97-99	96-98	$p < 0.001$
GeneXpert × Culture	91%	97%	87%	98%	96%	86%
CI	86-94	96-98	82-91	97-99	95-97	$p < 0.001$
cDSSM × Gene Xpert	69%	99%	95%	93%	93%	76%
CI	63-74	98-99.6	91-97	92- 95	92-95	$p < 0.001$
BC-DSSM × GeneXpert	97%	92%	98%	87%	96%	87%
CI	96-98	88-95	97-99	80-91	95-97	$p < 0.001$
cDSSM × BC-DSSM	100%	95%	76.5%	100%	96%	84%
CI	98-100	94-96	71-81	99.7-100	95-97	$p < 0.001$

cDSSM: direct sputum smear microscopy by conventional method; BC-DSSM: bleach-concentrated processed direct sputum smear microscopy; GeneXpert: rapid molecular assay; PPV: positive predictive value; NPV: negative predictive value; CI: 95% confidence interval, inferior-superior.

samples (Table 2). With regard to the 1,332 samples analyzed by culture and GeneXpert® MTB/RIF, 212 (16%) were positive and 1,066 (80%) negative by the two methods; 22 culture-positive samples were GeneXpert® MTB/RIF negative and 32 culture-negative samples were Xpert® MTB/RIF positive. In total, there were 234 culture-positive samples and 244 Xpert® MTB/RIF positive samples. For the calculation of the accuracy, Xpert® MTB/RIF positive/culture-negative samples were considered false-negative. The Xpert® MTB/RIF test was inconclusive in one of the 235 culture-positive samples, and the remaining 234 cultures were excluded from the comparison.

In the comparison of 1,346 samples analyzed by direct and processed sputum smear with Xpert® MTB/RIF, 255 (19%) were Xpert® MTB/RIF positive. From the 255 Xpert® MTB/RIF positive samples, 176 (69%) were positive and 79 (31%) negative (false-negative) by direct smear, 223 (87%) were positive and 32 (13%) were negative (false-negative) by processed sputum smear. Nine from the 1,091 (0.008%) Xpert® MTB/RIF negative samples were positive by direct and processed sputum smear. Among the 255 Xpert® MTB/RIF positive samples there were 47 (18%) more positive by processed than by direct sputum smear.

In the comparison within direct and processed sputum smear among 1,348 samples, 186 (13.8%) were positive by direct smear and 243 (18%) by processed sputum smear. There was an agreement between the negative results for AFB in 1,105 (82%) samples. There were 57 more positive by the processed sputum smear than by direct sputum smear, which corresponds to a 31% increase of positivity. Sensitivity, specificity, PPV, NPV, precision and a 95% confidence interval (CI) are shown in Table 3. The sensitivity, specificity, and agreement between the tests were satisfactory and the Kappa index above 50% (Table 3). In the analysis of the accuracy of the methods evaluated all the results were statistically significant.

DISCUSSION

The PTB patients are the more important disseminators of the infection in the community. Therefore, identifying and treating the carriers is the first step for controlling the disease as well as interrupting inter-human transmission[2].

Technological advances have provided automated rapid tests for laboratory diagnosis of pulmonary tuberculosis with high sensitivity, specificity, rapidity besides drug resistance information[4, 5, 13].

Even though poorly sensitive, the conventional direct sputum smear microscopy is still a valuable method for the detection of active PTB, especially in developing countries. The sensitivity of the direct sputum smear is highly improved accordingly the appropriate laboratory standards and the skills of the professional[2]. Aiming to improve the conventional direct sputum smear microscopy performance, several attempts have been made by using chemical treatment, such as the NaClO at different concentrations and times followed by centrifugation or spontaneous sedimentation with different results[6-9, 13].

In this study, sputum smear microscopy of bleach-concentrated specimens from patients exhibiting signs and symptoms of pulmonary tuberculosis was evaluated and compared to the conventional sputum smear microscopy, culture, and Xpert® MTB/

RIF system tests. From 235 (18%) culture-positive samples (true positive) the conventional sputum smear microscopy detected AFB in only 171 (73%). Thus, based only on direct sputum smear diagnosis, 27% of patients with active pulmonary tuberculosis would be categorized as negative for AFB. Estimating that one carrier can transmit the infection to 10 to 15 individuals by direct contact, after one year, another 600 more individuals would be infected[2].

Regarding the BC-DSSM results from the positive culture samples, there was an agreement in the majority (81%) of them. However, the positivity of the bleach-concentrated samples was lower than that from the cultures, considered as the gold standard test. On the other hand, BC-DSSM was able to detect *Mtb* from 22 pulmonary tuberculosis cases whose culture samples were negative. This disagreement could be due to the sputum decontamination procedure. For culture inoculation, the sputum is previously treated with 4% NaOH and the alkalinity impairs the viability and the growth of *Mtb* in the culture medium. Furthermore, only a very small aliquot of sputum is used for the Ogawa-Kudoh culture method in which a sputum impregnated swab is inoculated directly on the surface of the medium[14]. Despite that, the Ogawa-Kudoh medium has high sensitivity and specificity when compared to other *Mycobacterium* culture media[14, 15]. The microscopy detection of AFB among the culture-negative samples could be misleading due to the presence of non-tuberculous AFB of the *Mtb* complex (MtbC) that does not grow on the specific medium for the *Mtb*[10].

The negative results by BC-DSSM on samples of 44 positive culture samples are not surprising. As the cultures were performed prior to the direct and bleach-concentrated sputum smears, the possibility that the bacilli-richer portions were consumed is not ruled out, resulting in negative smear microscopy. Still compared to the culture, BC-DSSM positivity was 9% higher than that of direct sputum smear. In the analysis of accuracy, by adding the 22 positive BC-DSSM cases into the culture-negative samples, the bleach-concentrated sputum smear detected AFB in 213 from 1,332 analyzed samples or 16%, which is very close to the culture results (18%).

Concerning the accuracy, the culture performance was better than those of direct and bleach-concentrated sputum smear, but the BC-DSSM performance was better than that of the conventional direct sputum smear. There was disagreement in 22 culture-positive samples that were Xpert® MTB/RIF negative. As discussed above, this could also be misleading due to the presence of non-tuberculous AFB of the MtbC displaying the same morphology and dyeing characteristics, but which are not detectable by Xpert® MTB/RIF system[4].

For the determination of the accuracy, the 32 Xpert® MTB/RIF positive and culture-negative samples were considered false-negative. A positive Xpert® MTB/RIF result does confirm the presence of *Mtb* in the sample. However, this does not grant they are alive since the polymerase chain reaction (PCR) identifies the DNA from viable and non-viable microorganisms[4]. Therefore, the Xpert® MTB/RIF system accuracy was superior to that of the culture. Considering Xpert® MTB/RIF as the gold standard, the sensitivity of the culture was 96%.

In the analysis of the accuracy of the methods evaluated, all the results were statistically significant proving that they were not randomly positive or negative and confirming that there is a tendency for these results. The sensitivity, specificity, and agreement between the tests were satisfactory and the Kappa index was above 50% (Table 3).

Using conventional direct sputum smear as the standard for the calculations of the accuracy and the increment of the positivity, the BC-DSSM reproduced all positive results from the direct sputum smear. That is, no positive direct smear was negative by a processed sputum smear. Furthermore, there was an increment of 57 positive processed sputum smears among the negative direct smear samples. The rationale is that the step of the concentration of the bacilli increases the probability to be positive. When comparing the direct and the bleach-concentrated sputum smear techniques with Xpert® MTB/RIF, there were both Xpert® MTB/RIF negative and the BC-DSSM positive and the opposite. This could also be due to the presence of other non-tuberculous AFB of the MtbC with the morphology and dyeing characteristics of the *Mtb* but not detected by the Xpert® MTB/RIF[10, 11].

Previous studies aiming to improve the performance of the conventional direct sputum smear by chemical treatment of the sputum had quite varied results[6-9]. Our study showed 9% increased positivity regarding the conventional direct sputum smear by the treatment of the samples with commercial sodium hypochlorite. The bleach-concentrated sputum smear and culture had a comparable diagnostic accuracy. This is a very good improvement towards the identification of PTB cases missed by the conventional direct sputum smear. Another advantage of the BC-DSSM method is the increased safety for laboratory professionals[16, 17]. The NaClO impairs the Mtb viability and so, reduces the risk of contamination by aerosols generation. Furthermore, the NaClO promotes the dissolution of particles from the sputum (mucus, saliva), favoring the spontaneous precipitation of the *Mtb*, while maintaining its morphology and dyeing properties. The digestion of the particles from the sputum grants a cleaner microscopy field, which favors bacterial identification[10, 18].

As a drawback, NaClO digestion hampers the adherence of the smear to the slide, which can be easily washed away during staining[10]. In our work, this problem was circumvented by decreasing the fuchsin staining time step for 3 minutes, without causing bias to the staining results.

Finally, the chemical treatment and spontaneous sedimentation of the sputum not only preserves the advantages of the conventional direct smear but also considerably increases the identification of AFB in negative direct smear samples. Due to the simplicity of the procedure, which does not require the use of centrifuges, and the easy access and low-cost of the chemical, it is feasible to implement in laboratories of the basic health units.

The bleach-concentrated sputum smear microscopy procedure strengthens biosafety with the reduction of the risks of aerial contamination by *Mtb* and is more accurate compared to direct sputum smear allowing higher detection of positive cases. The procedure may be particularly useful for paucibacillary individuals like children or HIV/sexually transmitted diseases (STD) patients. Furthermore, in poverty areas, where more advanced technologies are not available, the implementation of the procedure in the PTB diagnostic routine is highly suitable.

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CONFLICTS OF INTEREST

None declared.

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