

## Is frontloaded sputum microscopy an option in active tuberculosis case finding?

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### SUMMARY

**SETTING:** Active tuberculosis (TB) case finding (ACF) in Phnom Penh, Cambodia using light-emitting diode fluorescence microscopy (FM).

**OBJECTIVE:** To evaluate the smear-positive yield of frontloaded (same-day) smear microscopy in ACF.

**DESIGN:** All presumptive TB cases screened through ACF were asked to provide three sputum specimens: two spot specimens on Day 1 and a morning specimen on Day 2 (spot-spot-morning, SSM). Laboratory technicians blinded to previous results read the smears using FM. We considered only SSM series with at least one positive smear to calculate the proportion of TB cases missed and to determine the difference between the spot-spot (SS) and spot-morning (SM) approach.

**RESULTS:** Of 4616 presumptive TB patients enrolled, 3306 provided three sputum samples. Of 2957 (89.4%)

who followed the SSM approach, 188 (6.4%) were smear-positive: 177 on SM and 160 on SS. The incremental yield of the second sputum sample was 18.1% for SM vs. 9.4% for SS. Relative to any smear-positive case detected by SSM, 28/188 (14.9%, 95%CI 10.1–20.8) TB cases would be missed by SS vs. 11/188 (5.9%, 95%CI 3.0–10.2) by SM. The difference in the proportion of missed TB patients was 9.0% ( $P=0.006$ ).

**CONCLUSION:** ACF frontloaded sputum microscopy is inferior in terms of smear-positive yield: the SS approach would have missed a significant proportion of smear-positive TB.

**KEY WORDS:** same-day diagnosis; smear microscopy; operational research; Cambodia; LED fluorescence microscopy

DESPITE RECENT ADVANCES, smear microscopy remains the key to tuberculosis (TB) diagnosis in many developing countries because it is rapid, affordable and widely available. Although using light emitting diode (LED) fluorescence microscopy (FM) improves yield, the technique lacks sensitivity. Furthermore, the process is highly inefficient. Many national TB programmes (NTPs) require microscopic examination of three sputum samples collected over 2 (or sometimes 3) days, following a spot-morning-spot (SMS) strategy whereby patients provide one sputum specimen at the time of consultation, one early morning specimen at home the following day and a third specimen on visiting the service to return the morning specimen. This requires repeated visits to return sputum samples and/or collect results, sometimes resulting in a high number of patients abandoning the diagnostic process.<sup>1</sup>

An earlier recommendation from the World Health

Organization (WHO) to reduce the number of specimens from three to two per series was justified by increased efficiency.<sup>2</sup> A systematic review confirmed the low additional yield of a third sputum smear in the SMS strategy:<sup>3,2</sup> the first two sputum specimens identified 95–98% of smear-positive cases from three specimens. Moreover, two specimens could lead to an improvement in the quality of smear reading by reducing the workload on already overburdened laboratory technicians and result in higher yield, as suggested by one study.<sup>4</sup> In 2010, the WHO recommended ‘frontloaded’ (i.e., collection, reading and reporting of two sputum specimens on the same day) microscopy under certain conditions.<sup>5</sup> There is no doubt that, if properly implemented, this strategy has many benefits for presumptive TB patients, with reduced cost, earlier exclusion or diagnosis of TB, as well as the NTP and the community in general, with potentially fewer smear-

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positive drop outs on the diagnostic and therapeutic pathway.

Several studies have reported frontloading to be as sensitive as smear microscopy using 2-day specimen collection including a morning sample.<sup>6–9</sup> However, as suggested in three recent studies from India, this approach may not be applicable to all settings.<sup>10–12</sup> Morning samples reportedly have a higher yield for smear positivity than spot samples,<sup>4,13,14</sup> and, as reported by Andrews et al., the collection of a morning sample might be of particular advantage if the disease is limited and of lower smear grade.<sup>13</sup> For ACF programmes, which aim for early—and therefore more paucibacillary—detection of TB, including a morning sample may be worth considering despite the logistic constraints.

These conflicting reports show that the role of frontloading needs to be clarified further, and in particular whether it is suitable for populations with high proportions of paucibacillary disease (severe immune depression in people living with human immunodeficiency virus [HIV], children, early case detection). In the present study, we aimed to evaluate the feasibility and yield of frontloaded sputum microscopy in community-based active TB case finding (ACF) in Cambodia.

## METHODS

### *Ethics considerations*

Ethics approval was granted by the National Ethics Committee for Health Research, Phnom Penh, Cambodia, the Institutional Review Board at the Institute of Tropical Medicine and the Ethics Committee of the University Hospital, Antwerp, Belgium. We also obtained permission from the NTP, the Municipality of Phnom Penh and the local health authorities. All participants provided written informed consent.

### *Study site and setting*

Cambodia, a low-income country in South-East Asia, has one of the highest TB prevalences in the world, with a weighted prevalence (bacteriologically positive) of 831 cases per 100 000 population<sup>15</sup> and a cumulative incidence of 411/100 000 in 2012.<sup>16</sup>

The Sihanouk Hospital Centre of HOPE (SHCH) functions as a tertiary referral hospital for TB in the capital city of Phnom Penh. The hospital's Mycobacteriology Laboratory performs the following diagnostic tests: bright field microscopy and FM, Xpert® MTB/RIF testing (Cepheid, Sunnyvale, CA, USA), conventional culture on Löwenstein-Jensen (LJ) medium and drug susceptibility testing (DST) using the proportion method.

Between 9 February 2012 and 31 March 2013, we prospectively collected data from presumptive TB cases in a community-based ACF project conducted

by SHCH targeting socio-economically vulnerable individuals, as previously described.<sup>17</sup> TB workers collected sputum specimens at home from symptomatic individuals (defined as any cough, unintentional weight loss, fever, night sweats or haemoptysis). We requested three sputum specimens, following a spot-spot-morning (SSM) collection strategy: two spot specimens, ideally within approximately 1-h interval, were collected on Day 1, followed by a morning specimen on Day 2. In the laboratory, the sputum specimens were stained with auramine-O and directly examined for acid-fast bacilli (AFB) using LED fluorescence microscopes (iLED Primostar, Zeiss, Oberkochen, Germany). Other sputum investigations not related to this study, such as Xpert and culture, were performed per protocol.<sup>17</sup>

### *Data collection*

We prospectively analysed all sputum microscopy results collected for the ACF project among individuals aged  $\geq 15$  years and whose specimens were sent to the SHCH. All smears were read using LED FM by laboratory technicians blinded to the previous smear results and specimen type. Smear positivity was defined as at least one smear containing at least one AFB.<sup>18</sup> When more than one result was positive, the highest smear grade was taken. All smears were graded according to the WHO/International Union against Tuberculosis and Lung Disease scale.<sup>19</sup> The NTP ensured quarterly external quality assessments (blinded rechecking). The laboratory's microscopy performance was good: there were no discrepant results reported over the study period.

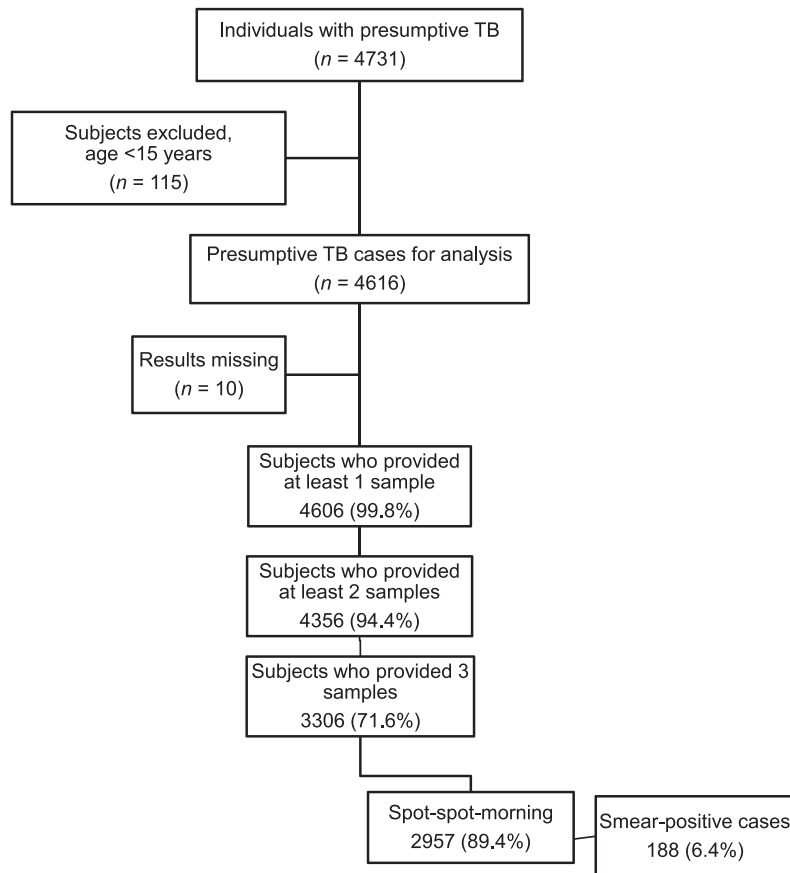
The TB workers used a structured form to collect the following variables: age, sex, referring health centre, date of sputum collection and result, sputum timing (spot vs. morning) and microscopy result. A team of data entry clerks performed single-data entry using Access software (Microsoft Access 2010, Microsoft, Redmond, WA, USA).

### *Statistical analysis*

We considered only specimens from individuals who submitted three sputum samples using the SSM strategy, with at least one positive smear, to calculate the theoretical proportion and 95% confidence intervals (95% CIs) of TB cases missed if the spot-spot (SS) and the spot-morning (SM) approach were used in our population and to determine the difference between SS and SM approaches using McNemar's test. All analyses were performed using STATA software version 10.0 (Stata Corp, College Station, TX, USA).

## RESULTS

Of the 4616 presumptive TB patients enrolled, 2053 (45%) were male and the median age was 49 years



**Figure** Flow diagram of sputum collection from presumptive TB cases. TB = tuberculosis.

(interquartile range 33–62). The Figure shows the flow diagram for sputum collection in this operational setting. The microscopy results of 10 patients could not be traced. All of the remaining 4606 (99.8%) provided at least one sputum specimen, 4356 (94.4%) provided at least two and 3306 (71.6%) provided three. While SSM was the per protocol collection strategy, this was feasible for only 2957 of the 4297 (68.8%) participants whose strategy was known. Alternative sputum collection strategies for individuals unable to follow the instructions for any reason are listed in Table 1. Taking into account only three complete specimens following the SSM method, 188 (6.4%) were smear-positive.

Table 2 shows the smear-positive yield of the SS and SM collection approaches. If the SM strategy had been followed, 177/2957 (6.0%) smear-positive TB cases would have been diagnosed, compared to 160/2957 (5.4%) for the SS strategy. The sputum smear positivity grading was comparable for both approaches. The incremental yield of the second sputum specimen was proportionally 90% higher when collecting one spot and one morning specimen compared to two spot specimens, with 18% (32 cases) vs. 9% (15 cases).

Table 3 shows the number of TB cases missed. Of

any smear-positive case detected using the SSM method, 28/188 (14.9%, 95% CI 10.1–20.8) TB cases would be missed using SS vs. 11/188 (5.9%, 95% CI 3.0–10.2,  $P = 0.006$ ) using SM. The difference in the proportion of missed TB patients was 9.0% (95% CI 2.7–15.4).

## DISCUSSION

This is the first study to evaluate the feasibility and smear-positive yield of SS (frontloaded) sputum microscopy in comparison with an SM approach in the context of ACF. Our findings show that a two-specimen strategy would be rewarding in ACF provided an early morning specimen is collected. Omitting the morning specimen would result in 18% of TB cases being missed. In terms of maximum yield, frontloaded microscopy might not be applicable to ACF, unless an SM strategy is too demanding for community workers.

The WHO recommends frontloaded SS microscopy in countries that have successfully implemented the two-specimen strategy and documented good-quality smear microscopy results. The strategy is particularly recommended in settings where patients are likely to default from the diagnostic process.<sup>5</sup> In our field study, nearly one in three presumptive TB cases failed

**Table 1** Number of patients who provided sputum samples by specimen collection strategy (spot-spot-morning was the per protocol approach) and smear-positive yield

Specimen collection strategy	Patients providing sputum <i>n</i>	Smear-positive result <i>n</i>
Total*	4606	278
Three specimens obtained, <i>n</i> (%)	3306 (71.8)	205
Spot-spot-morning	2957	188
Morning-spot-spot	32	3
Spot-morning-morning	24	3
Morning-spot-morning	14	0
Spot-morning-spot	12	2
Morning-morning-morning	9	0
Morning-morning-spot	8	1
Spot-spot-spot	1	0
Unknown	249	8
Two specimens obtained, <i>n</i> (%)	1050 (22.8)	64
Spot-spot	595	36
Spot-morning	291	17
Morning-spot	61	4
Morning-morning	56	6
Unknown	47	1
One specimen obtained, <i>n</i> (%)	250 (5.4)	9
Spot	166	1
Morning	71	8
Unknown	13	0

\* TB workers were instructed to collect sputum according to the spot-spot-morning strategy. Other specimen collection strategies were allowed if presumptive TB patients were unable to follow the instructions for any reason, as this study was embedded in an operational case-finding project.  
TB = tuberculosis.

to provide three sputum specimens. Surprisingly, the proportion is as high as 21–32% of presumptive TB cases not returning for the third specimen in passive case finding in certain settings.<sup>20,21</sup> Whereas repeated visits to the health centre are not a concern in ACF when sputum is collected in the community, defaulting from the diagnostic pathway is common for other reasons, such as unavailability of presumptive cases

**Table 2** Comparison of spot-morning vs. spot-spot sputum collection strategies in terms of yield and smear grading from active case finding in Cambodia (only specimens from individuals who submitted three specimens following the spot-spot-morning strategy with at least one positive smear were included)

	Sputum collection strategy	
	Spot-morning <i>n</i> (%)	Spot-spot <i>n</i> (%)
Total	2957	2957
Smear-positive results	177 (6.0)	160 (5.4)
Positive smear grading*		
Scanty	67 (38)	61 (38)
1+	33 (19)	25 (16)
2+	24 (14)	24 (15)
3+	53 (30)	50 (31)
Incremental yield of the second sample <sup>†</sup>	32 (18)	15 (9)

\* In case of two or more positive smears, the highest grade was considered.

<sup>†</sup> All individuals with a first negative spot and a second positive spot or morning specimen.

**Table 3** Number of smear-positive TB cases missed by the spot-spot or spot-morning collection strategies out of the total 188 smear-positive TB cases diagnosed using the spot-spot-morning method in active case finding in Cambodia

Spot-spot strategy	Spot-morning strategy		Total
	Positive smear	Negative smear	
Positive smear	149	11	160
Negative smear	28	2769	2797
Total	177	2780	2957

TB = tuberculosis.

(out at work) and the absence of a productive cough associated with less advanced disease. Reducing the recommended number of specimens from three to two therefore seems appropriate in community-based ACF. The two-specimen collection could also increase the effectiveness of community health workers and reduce the laboratory workload.<sup>2</sup>

Which samples are to be collected when considering a two-sputum screening strategy remains controversial in passive case finding, let alone in ACF. The WHO recommendation of frontloaded SS microscopy is based on a systematic review by Davis et al. (including four major reports) comparing the diagnostic accuracy of same-day (frontloaded) microscopy with standard microscopy.<sup>1,7,9,22,23</sup> They concluded that smear microscopy performed on two sputum specimens collected on the spot in 1 day was as accurate as microscopy of specimens collected over 2 days, irrespective of whether two or three smears are read. All included studies were on passive TB case findings, and were from a limited number of settings.

Since this publication, several operational research studies have been conducted on same-day microscopy in passive case finding, often with conflicting results.<sup>6,10–12,24,25</sup> Reports from Uganda, Libya and India supported frontloading,<sup>6,24,25</sup> while three other studies from India did not.<sup>10–12</sup> Although different case-finding strategies were used, our findings are in line with a recently published study from India. Nayak et al. compared the SS vs. SM approaches and reported that 8% of TB cases would be missed using the same-day strategy.<sup>12</sup> The difference is even more pronounced in our study, with 18.1% missed with SS vs. 9.4% with SM.

Sputum quality is important for optimal smear yield.<sup>26</sup> Spontaneous expectoration of two good-quality sputum specimens on the spot can be challenging for patients in the early stages of disease who are targeted by ACF. Furthermore, sputum specimens of identified cases during ACF have a lower bacillary load than passively found cases.<sup>27–29</sup> Similar conditions can be found in children or immunosuppressed (TB-HIV co-infection) patients who often present with non-cavitary disease. In our series, the relatively high proportion of scanty smears in spot as well as morning specimens reflects early



presentation. While we did not systematically record sputum appearance, many specimens were salivary (T Kim, personal communication). In the few studies evaluating frontloading that comment on sputum quality, a higher proportion of spot specimens were salivary;<sup>22,11</sup> this proportion was lower (24% vs. 38%) in the report favouring the SS approach, implying more advanced disease.<sup>22</sup>

The higher positive yield of morning samples (or samples collected overnight) compared to spot samples has been shown repeatedly.<sup>13,30</sup> Andrews and Radhakrishna showed that the difference between spot and morning specimens is of particular importance in non-cavitary disease, as is common in ACF, with 30% vs. 46% positives detected by smear and 63% vs. 80% using culture.<sup>13</sup> Despite the lack of comment on smear grading (and hence the disease stage) of actively found TB among prisoners in Bangladesh, Islam et al. also found a significantly higher smear-positive yield in morning vs. spot specimens.<sup>31</sup> A strategy of collecting two successive morning samples to be delivered simultaneously to the health centre was recommended by Van Deun et al. more than a decade ago as the most effective and also the most highly efficient strategy.<sup>30</sup> This approach would be more convenient for patients having to produce sputum when it is most easy to expectorate and would remove the logistic constraints of repeated visits for both patients and health workers; health care workers would need to spend less time encouraging patients to produce good quality specimens, and laboratory technicians would find themselves spending less time screening smears for AFB, given the higher yield of morning specimens. Applying this strategy would be in line with the WHO recommendation to collect two specimens in 1 day, as it would also allow same-day reporting. For ACF, this approach deserves particular consideration and may well be the way forward.

There are a number of limitations to our study. First, although we designed our study to reflect operational realities as much as possible, our analysis results from patients who provided three samples, which may have created bias. Individuals with more advanced disease might be more motivated and have less difficulty providing three samples. However, the smear grading results refute this assumption. Furthermore, a similar incremental yield and proportion of missed smear-positive case rates were obtained when a more pragmatic approach, including all patients who submitted at least two (SS or SM) samples, was adopted (data not shown). Second, specimens included in the study were read by laboratory technicians at a single non-governmental hospital. Our laboratory technicians spend more time reading smears and tend to identify more scanty smears than public laboratories, where these few bacilli could be missed.<sup>32</sup> This indicates that there is

all the more reason to collect sputum specimens with the highest likelihood of smear positivity in this setting. Third, the yield of the different two-specimen approaches was not directly compared, but was deduced from reading any of three specimens collected. Workload and fatigue have a huge impact on smear quality reading. However, as this is a drawback of all studies evaluating frontloading, it is unlikely to have affected our results differently. Ideally, future studies should perform direct comparisons of the smear collection methods.

In conclusion, our study findings show that frontloaded sputum microscopy is inferior in terms of smear-positive yield in ACF. The SS approach would have missed a significant proportion of smear-positive TB cases compared to an approach including a morning specimen. As Cambodia is one of the pioneers in active TB case finding, our data can inform national policy regarding sputum collection for the various ongoing and future ACF projects. The trade off in the maximum yield and efficiency of collection strategy might play an important role in the decision. Furthermore, these data contribute to the growing evidence that the same-day spot-spot microscopy recommendation may not be applicable to every context. However, same-day collection of two consecutive morning samples might be the way forward in ACF.

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#### References

- 1 Davis J L, Cattamanchi A, Cuevas L E, Hopewell P C, Steingart K R. Diagnostic accuracy of same-day microscopy versus standard microscopy for pulmonary tuberculosis: a systematic review and meta-analysis. *Lancet Infect Dis* 2013; 13: 147–154.
- 2 Bonnet M, Ramsay A, Gagnidze L, Githui W, Guerin P J, Varaine F. Reducing the number of sputum samples examined and thresholds for positivity: an opportunity to optimise smear microscopy. *Int J Tuberc Lung Dis* 2007; 11: 953–958.
- 3 Mase S R, Ramsay A, Ng V et al. Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review. *Int J Tuberc Lung Dis* 2007; 11: 485–495.
- 4 Noeske J, Dopico E, Torrea G, Wang H, Van Deun A. Two vs. three sputum samples for microscopic detection of tuberculosis in a high HIV prevalence population. *Int J Tuberc Lung Dis* 2009; 13: 842–847.
- 5 World Health Organization. Same-day diagnosis of tuberculosis by microscopy. Policy statement. WHO/HTM/TB/2011.7. Geneva, Switzerland: WHO, 2011.

- 6 Chandra T J, Raj R S, Sharma Y V. Same day sputum smear microscopy approach with modified ZN staining for the diagnosis of pulmonary tuberculosis in a microscopy centre at Rajahmundry. *Indian J Med Microbiol* 2014; 32: 153–156.
- 7 Cuevas L E, Yassin M A, Al-Sonboli N, et al. A multi-country non-inferiority cluster randomized trial of frontloaded smear microscopy for the diagnosis of pulmonary tuberculosis. *PLOS MED* 2011; 8: e1000443.
- 8 Hirao S, Yassin M A, Khamofu H G, et al. Same-day smears in the diagnosis of tuberculosis. *Trop Med Int Health* 2007; 12: 1459–1463.
- 9 Ramsay A, Yassin M A, Cambanis A, et al. Front-loading sputum microscopy services: an opportunity to optimise smear-based case detection of tuberculosis in high prevalence countries. *J Trop Med* 2009; 2009: 398 767.
- 10 Myneedu V P, Verma A K, Sharma P P, Behera D. A pilot study of same day sputum smear examination, its feasibility and usefulness in diagnosis of pulmonary TB. *Indian J Tuberc* 2011; 58: 160–167.
- 11 Nayak P, Kumar A M, Claassens M et al. Comparing same-day sputum microscopy with conventional sputum microscopy for the diagnosis of tuberculosis, Chhattisgarh, India. *PLOS ONE* 2013; 8: e74964.
- 12 Nayak P, Kumar A M, Agrawal T K, Chandraker S, Nair S A. Same-day light-emitting diode fluorescence microscopy for the diagnosis of tuberculosis in Chhattisgarh, India. *Int J Tuberc Lung Dis* 2014; 18: 666–670.
- 13 Andrews R H, Radhakrishna S. A comparison of two methods of sputum collection in the diagnosis of pulmonary tuberculosis. *Tubercle* 1959; 40: 155–162.
- 14 Van Deun A, Salim A H, Cooreman E, et al. Scanty AFB smears: what's in a name? *Int J Tuberc Lung Dis* 2004; 8: 816–823.
- 15 Eang M T, Okada K, Yamada N, et al. Cross-sectional studies of tuberculosis prevalence in Cambodia between 2002 and 2011. *Bull World Health Organ* 2014; 92: 573–581.
- 16 World Health Organization. Global tuberculosis report 2013. WHO/HTM/TB/2013.11. Geneva, Switzerland: WHO, 2013.
- 17 Lorent N, Choun K, Thai S, et al. Community-based active tuberculosis case finding in poor urban settlements of Phnom Penh, Cambodia: a feasible and effective strategy. *PLOS ONE* 2014; 9: e92754.
- 18 World Health Organization. Treatment for tuberculosis guidelines. 4<sup>th</sup> ed. WHO/HTM/TB/2009.420. Geneva, Switzerland: WHO, 2009.
- 19 Ait-Khaled N, Alarcon E, Armengol R, et al. Management of tuberculosis: a guide to the essentials of good practice. 6<sup>th</sup> ed. Paris, France: International Union Against Tuberculosis and Lung Disease, 2010.
- 20 den Boon S, Semitala F, Cattamanchi A, et al. Impact of patient drop-out on the effective sensitivity of smear microscopy strategies. *Am J Respir Crit Care Med* 2010; 181: A2258.
- 21 Harries A D, Mphasa N B, Mundy C, Banerjee A, Kwanjana J H, Salaniponi F M. Screening tuberculosis suspects using two sputum smears. *Int J Tuberc Lung Dis* 2000; 4: 36–40.
- 22 Cattamanchi A, Huang L, Worodria W, et al. Integrated strategies to optimize sputum smear microscopy: a prospective observational study. *Am J Respir Crit Care Med* 2011; 183: 547–551.
- 23 Cuevas L E, Al-Sonboli N, Lawson L, et al. LED fluorescence microscopy for the diagnosis of pulmonary tuberculosis: a multi-country cross-sectional evaluation. *PLOS Med* 2011; 8: e1001057.
- 24 Gammo M, Lamaric W, Hadida M, et al. Front-loaded smear microscopy for the diagnosis of pulmonary TB in Tripoli, Libya. *Trans R Soc Trop Med Hyg* 2013; 107: 137–139.
- 25 Mirembe P, Kalyango J N, Worodria W, Mugerwa H, Nakakawa E, Asiimwe B B. Performance of frontloading for smear microscopy in the diagnosis of pulmonary tuberculosis: a cross-sectional study at a referral hospital in Uganda. *PLOS ONE* 2012; 7: e48531.
- 26 Alisjahbana B, van Crevel R, Danusantoso H, et al. Better patient instruction for sputum sampling can improve microscopic tuberculosis diagnosis. *Int J Tuberc Lung Dis* 2005; 9: 814–817.
- 27 den Boon S, Verver S, Lombard C J, et al. Comparison of symptoms and treatment outcomes between actively and passively detected tuberculosis cases: the additional value of active case finding. *Epidemiol Infect* 2008; 136: 1342–1349.
- 28 Eang M T, Satha P, Yadav R P, et al. Early detection of tuberculosis through community-based active case finding in Cambodia. *BMC Public Health* 2012; 12: 469.
- 29 van't Hoog A H, Laserson K F, Githui W A et al. High prevalence of pulmonary tuberculosis and inadequate case finding in rural western Kenya. *Am J Respir Crit Care Med* 2011; 183: 1245–1253.
- 30 Van Deun A, Salim A H, Cooreman E, et al. Optimal tuberculosis case detection by direct sputum smear microscopy: how much better is more? *Int J Tuberc Lung Dis* 2002; 6: 222–230.
- 31 Islam M R, Khatun R, Uddin M K, et al. Yield of two consecutive sputum specimens for the effective diagnosis of pulmonary tuberculosis. *PLOS ONE* 2013; 8: e67678.
- 31 World Health Organization Western Pacific Region. The Kingdom of Cambodia: joint review of the National TB Programme, 2012. Phnom Penh, Cambodia: National Tuberculosis and Leprosy Programme, National Center for Tuberculosis & Leprosy Control (CENAT), 2012.

## RESUME

**CONTEXTE :** Recherche active de cas de tuberculose (ACF) à Phnom Penh, Cambodge, grâce à la microscopie à fluorescence (FM) à diode électroluminescente.

**OBJECTIF :** Evaluer le rendement en termes de frottis positif de la microscopie de frottis cueillis le même jour dans le cadre de l'ACF.

**SCHEMA :** Tous les cas présumés de tuberculose (TB) dépistés par ACF ont été invités à fournir trois échantillons de crachats, deux sur place le premier jour et un troisième le matin du deuxième jour (SSM). Les techniciens de laboratoire, ignorants des résultats préalables, ont lu les frottis avec un microscope FM. Nous avons tenu compte seulement les séries SSM avec au moins un frottis positif pour calculer la proportion de cas de TB manqués et déterminer la différence entre l'approche spot-spot (SS) et spot-matin (SM).

**RÉSULTATS :** Sur 4616 patients suspects de TB enrôlés, 3306 ont fourni trois crachats. Chez les 2957 (89,4%) avec un recueil SSM, 188 (6,4%) étaient frottis positifs : 177 après SM et 160 après SS. Le gain supplémentaire généré par le deuxième crachat était de 18,1% pour le recueil SM contre 9,4% pour le recueil SS. Par rapport aux cas à frottis positif détectés par SSM, 28/188 (14,9% ; IC95% 10,1–20,8) cas de TB auraient été manqués par SS contre 11/188 (5,9% ; 95%IC 3,0–10,2) par SM. La différence de proportion de cas de patients tuberculeux manqués était de 9,0% ( $P=0,006$ ).

**CONCLUSION :** La recherche active par microscopie de crachats cueillis le même jour initiale est inférieure en termes de rendement de frottis positifs : l'approche SS aurait manqué une proportion significative de TB à frottis positif.

## RESUMEN

**MARCO DE REFERENCIA:** La estrategia de búsqueda activa de casos (ACF) de tuberculosis (TB) en Phnom Penh, Camboya, mediante la microscopia de fluorescencia (FM) con indicadores luminosos.

**OBJETIVO:** Evaluar el rendimiento de baciloscopias positivas alcanzado con el examen microscópico de una muestra de esputo recogida inmediatamente durante la consulta en el contexto de la ACF de TB.

**MÉTODO:** Se solicitó a todos los pacientes con presunción clínica de TB, examinados en el marco de una ACF, que aportaran tres muestras de esputo, dos inmediatas en el día 1 y una muestra matinal en el día 2 (serie SSM). Los auxiliares de laboratorio, sin conocimiento de los resultados anteriores, leyeron los frotis en el FM. Se tuvieron en cuenta solo las series SSM de muestras de esputo que presentaron como mínimo una baciloscopia positiva, a fin de calcular la proporción de casos de TB pasados por alto y se determinó la diferencia entre series con dos muestras inmediatas (SS) y series con una muestra inmediata y una matinal (SM).

**RESULTADOS:** De los 4616 pacientes con presunción

clínica de TB participantes, 3306 aportaron tres muestras de esputo. De 2957 que suministraron una serie de muestras SSM (89,4%), 188 (6,4%) obtuvieron un resultado positivo de la baciloscopia; 177 fueron positivas con muestras SM, en comparación con 160 con muestras SS. El incremento diferencial de la segunda muestra fue 18,1% con una segunda muestra matinal comparado con 9,4% con la segunda muestra inmediata. Con respecto a todos los casos detectados con la serie de muestras SSM, las series SS habrían pasado por alto 28 de los 188 casos de TB (14,9%; IC95% 10,1–20,8), en comparación con 11 de los 188 con las series SM (5,9%; IC95% 3,0–10,2). La diferencia en la proporción de casos pasados por alto fue 9,0% ( $P=0,006$ ).

**CONCLUSIÓN:** La ACF mediante la obtención de muestras inmediatas para baciloscopia aporta un menor rendimiento de resultados positivos; con la estrategia SS se habría pasado por alto una proporción considerable de casos de TB con baciloscopia positiva.