Are Two Sputum Samples Enough for Diagnosing Pulmonary Tuberculosis

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Objective: *To determine the optimum number of sputum specimens for smear and culture in the diagnosis of pulmonary tuberculosis.*
Material and Method: *A retrospective study was conducted in culture-positive pulmonary tuberculosis patients at Siriraj Hospital during April 2009 to October 2010. Number of sputum specimens and microbiological results were retrieved from the microbiologic laboratory. Positive yield and incremental yield of each sputum specimen were calculated.*
Results: *There were 401 patients during the study period, 153 (38.2%) had positive smear for acid-fast bacilli. Overall diagnostic yields of solid culture media and liquid culture media, were 72.1% and 95.3% respectively. Incremental of overall diagnostic yield from 1 to 2 and 2 to 3 sputum specimens were 8% and 6% respectively.*
Conclusion: *In place where a routinely combined smear and culture for every sputum sample submitted to the microbiologic laboratory, two specimens are sufficient for the diagnosis in nearly all pulmonary tuberculosis patients.*

Keywords: *Pulmonary tuberculosis, Sputum smear, Sputum culture*

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Tuberculosis remains one of the world's major causes of illness and death and Thailand is ranked 17th on the list of 22 high TB burden countries in 2009[1]. The early identification of acid-fast bacilli (AFB) from sputum smear and/or subsequent isolation of *Mycobacterium tuberculosis* (MTB) are the mainstay for the diagnosis of pulmonary tuberculosis (PTB). In a systematic review, the first sputum specimen for AFB smear was positive in 85.8% of the patients with PTB, and the incremental yield of the second and third specimens was only 11.9 and 2.3% respectively[2]. Recent study in those with HIV co-infection from Thailand and Vietnam, the third sputum smear added little to the diagnosis PTB (2%), but the third specimen for culture increased the result up to 10%[3].

The World Health Organization (WHO) recently recommends the number of specimens to be examined for screening of PTB cases can be reduced from 3 to 2 in places where a well-functioning external quality assurance (EQA) system exists and the workload is very high whilst human resources are limited[4]. WHO has also endorsed the use of liquid medium for culture and drug susceptibility testing because of its high sensitivity with less detection time.

In Siriraj Hospital, the microbiologic laboratory performs smear and culture in every sputum sample from patients suspected to have PTB. The authors aim to determine the optimum number of sputum smears and cultures to fulfill this diagnostic task.

Material and Method

The present study was conducted from April 2009 to October 2010 in patients aged more than 15 years who had positive sputum culture for MTB. All sputum specimens had been processed for acid-fast smear (Kinyoun), Lowenstein Jansen (L-J) solid medium culture and Mycobacterial Growth Indicator Tube (Becton Dickinson Microbiology System) liquid medium culture following standard protocol. Positive cultures were identified as MTB by means of real-time

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polymerase-chain reaction[5]. Demographic data, clinical background and consideration for treatment were reviewed from the medical records. The present study protocol was approved by the Siriraj Institutional Review Board.

Statistical analysis was performed using statistical software SPSS version 13.0 (SPSS Inc, Chicago, USA). The data were expressed as number (%) and mean ± standard deviation. Comparison of continuous data was performed using t-test. Value of $p < 0.05$ was considered to be statistically significant.

Because the patients submitted either 1 or 2 or 3 morning sputum samples without an identification of sequence, so the estimation of positive results in each sample was performed using random chance determined by probability. Positive first sample was defined as the positive 1 in 1 and 2 in 2 and 3 in 3 samples submission, plus half of the positive 1 in 2 samples submission, plus one-third of the positive 2 in 3 samples submission and plus one-third of the positive 1 in 3 samples submission. Positive second sample was defined as positive first sample plus half of the positive 1 in 2 samples submission, plus one-third of the positive 2 in 3 samples submission and plus one-third of the positive 1 in 3 samples submission.

Results

A total of 401 culture-positive pulmonary tuberculosis patients were enrolled, the mean age was 48 ± 19 years and 56% were male. For those whose HIV antibody has been tested, 8.8% had positive result. Concomitant extrapulmonary involvement was identified in 5.7% of the patients.

Acid-fast smear

The number of patients who submitted 1, 2, and 3 specimen for smears were 91, 65 and 245 accordingly (Table 1). Positive smears were identified in 38.2% of the patients. The overall diagnostic yield for smears was 38.2%. Positive yield of first and second samples were 84.3% and 92.8% respectively. Incremental yield of acid-fast smear from first to second and second to third samples were 8.5% and 7.2% respectively.

Solid culture

The number of patients who submitted 1, 2, and 3 specimen for solid culture were 101, 66 and 234 accordingly (Table 2). The overall diagnostic yield for solid culture was 72.1%. Positive yield of first and second samples were 78.5% and 90.7% respectively. Incremental yield of solid culture from first to second and second to third samples were 12.2% and 9.3%.

Liquid culture

The number of patients who submitted 1, 2, and 3 specimen for liquid culture were 101, 66 and 234 accordingly (Table 3). The overall diagnostic yield for liquid culture was 95.3%. Positive yields of first and second samples were 76.3% and 89.5% respectively. Incremental yield of liquid culture from first to second and second to third samples were 13.2% and 10.5%.

Overall culture

The overall positive yields of liquid and solid culture from first and second samples were 86% and 94%, respectively (Table 4). Incremental yields of overall culture from first to second and second to third samples were 8 and 6%.

Time to positive culture

Time to positive culture from liquid and solid media were 13.3 ± 7.6 and 31.1 ± 7.7 days respectively ($p < 0.001$).

Table 1. Number of patients who had positive acid-fast smear

No. of specimens	Number of positive smear (%)				Total
	0	1	2	3	
1	70	21	-	-	91
2	44	4	17	-	65
3	134	9	24	78	245
Total	248 (61.8)	34 (8.5)	41 (10.2)	78 (19.5)	401

* Positive yield of first sample = (21 + 17 + 78) + (4/2) + (24/3) + (9/3) = 129/153 x 100 = 84.3%, Positive yield of second sample = 129 + [(4/2) + (24/3) + (9/3)]/153 x 100 = 92.8%

Table 2. Number of patients who had positive solid culture

No. ofspecimens	Number of positive solid culture (%)				Total
	0	1	2	3	
1	47	54	-	-	101
2	15	16	35	-	66
3	50	32	49	103	234
Total	112 (27.9)	102 (25.4)	84 (20.9)	103 (25.7)	401

* Positive yield of first sample = (54 + 35 + 103) + (16/2) + (49/3) + (32/3) = 227/289 x 100 = 78.5%, Positive yield of second sample = 227 + [(16/2) + (49/3) + (32/3)] = 262/289 x 100 = 90.7%

Table 3. Number of patients who had positive liquid culture

No. of specimens	Number of positive liquid culture (%)				Total
	0	1	2	3	
1	10	91	-	-	101
2	0	21	45	-	66
3	9	37	83	105	234
Total	19 (4.7)	149 (37.2)	128 (31.9)	105 (26.2)	401

* Positive yield of first sample = (91 + 45 + 105) + (21/2) + (83/3) + (37/3) = 291.5/382 x 100 = 76.3%, Positive yield of second sample = 291.5 + [(21/2) + (83/3) + (37/3)] = 342/382 x 100 = 89.5%

Table 4. Number of patients who had overall positive culture

No. of specimens	Number of overall positive culture (%)			Total
	1	2	3	
1	101	-	-	101
2	15	51	-	66
3	28	45	161	234
Total	144 (36.0)	96 (23.9)	161 (40.1)	401

* Positive yield of first sample = (101 + 51 + 161) + (15/2) + (45/3) + (28/3) = 344.8/401 x 100 = 86%, Positive yield of second sample = 344.8 + [(15/2) + (45/3) + (28/3)] = 376.6/401 x 100 = 94%

Determination for treatment

Among 304 patients receiving treatment in Siriraj Hospital, treatment was commenced before the microbiological results in 132 patients (43.4%).

Discussion

Vital components of TB control are the early diagnosis and treatment for those suspected to have the disease. Much attention has been paid toward increasing the yield of various diagnostic tools for sputum sample, but little has been directed to the optimum number of specimen. In the evaluation of patients with at least 3 sputum specimens were collected, Nelson et al have demonstrated that the third or later specimen was the first positive smear and culture in 13 and 7% of the patients respectively[6]. These figures are comparable to the incremental yield of 7.2% from smear and 6% from culture of third specimen in the present study. The positive result of sputum smear in this report was only 38.2% and was nearly the same

as the present study in patients with HIV co-infection from Thailand and Vietnam (38%)[3]. These may result from the high detection power of current culture systems for less number of bacilli in the clinical specimen. Adoption of culture in addition to smear should be recommended to the higher health care facility where resources are affordable.

An application of two-specimen strategy can reduce laboratory workload and decrease burden for the patients. Although the prevalence of TB and HIV co-infection are high in Thailand as in some parts of the world, the third sputum specimen has also added little diagnostic value in these confined populations[3,7-9]. For those with high clinical suspicious PTB, anti-tuberculosis agents are usually started regardless of the smear results as demonstrated in nearly half of the patients in the present study. A third sputum sample should be reserved for previously treated patients where identification of MTB is essential. Other measures such as spot sputum collection and examination method to improve specimen submission while preserving the diagnostic yield have also been demonstrated[10,11].

The diagnostic yields of liquid and solid media in this study were 95.3 and 72.1% respectively and were in line with the results of 93 and 76.9% as shown by Idiogoras et al[12]. The time to positive culture from liquid medium was significantly shorted by half when compared with solid medium as has been described in the earlier studies[12,13]. But the solid media culture system could not be ignored, since 4.7% of the patients in the present study had positive results only from this system. In the authors experience, liquid media culture system also has a greater chance for bacterial contamination. Therefore both liquid and solid cultures should be performed together, especially in patients for whom mycobacterial identification is vital for clinicians.

The potential limitation of the present study resulted from completion of three specimen submission were established in about two-third of the patients. An absence of accurate data about the sequence of sputum specimens submitted to the laboratory was also another limitation. A future prospective study for the cost-effectiveness determination of a two-specimen approach should be conducted.

Conclusion

In place where routinely combined smears and cultures in every sputum sample submitted to the microbiologic laboratory, two consecutive specimens are sufficient for the diagnosis in nearly all of culture-positive pulmonary tuberculosis patients.

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Potential conflicts of interest

None.

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การส่งตรวจเสมหะสองครั้งเพียงพอในการวินิจฉัยวัณโรคปอดหรือไม่

นิธิพัฒน์ เจียรกุล, พิชญา เพชรบรม, ศุภร ฟุ้งลัดดา, สุทธิพล อุดมพันธุรัก

วัตถุประสงค์*: เพื่อประเมินจำนวนครั้งที่เหมาะสมของการส่งเสมหะเพื่อตรวจย้อมและเพาะเชื้อสำหรับการวินิจฉัยวัณโรคปอด*

วัสดุและวิธีการ*: ได้ทำการศึกษาแบบย้อนหลังในผู้ป่วยวัณโรคปอดที่เพาะเชื้อได้จากเสมหะของโรงพยาบาลศิริราช ระหว่างเดือนเมษายน พ.ศ. 2552 ถึงเดือนตุลาคม พ.ศ. 2553 โดยรวบรวมข้อมูลจำนวนครั้งของการส่งเสมหะและข้อมูลทางด้านจุลชีววิทยาจากห้องปฏิบัติการแล้วคำนวณหาการให้ผลบวกและการเพิ่มขึ้นของผลบวกจากเสมหะที่ส่งตรวจแต่ละครั้ง*

ผลการศึกษา*: มีผู้ป่วยทั้งหมด 401 คน ในช่วงเวลาดังกล่าวโดยตรวจพบเชื้อจากการย้อมสีทนกรดจำนวน 153 คน คิดเป็นร้อยละ 38.2 ผลรวมความสามารถในการวินิจฉัยด้วยการเพาะเชื้อในสารเลี้ยงเชื้อชนิดแข็งและชนิดเหลว คิดเป็นร้อยละ 72.1 และ 95.3 ตามลำดับ มีการเพิ่มขึ้นของผลรวมความสามารถในการวินิจฉัยจากการส่งตรวจเสมหะเพิ่มจาก 1 เป็น 2 ครั้งและจาก 2 เป็น 3 ครั้ง ที่ร้อยละ 8 และร้อยละ 6 ตามลำดับ*

สรุป*: ในสถานที่ที่ห้องปฏิบัติการทำการตรวจย้อมและเพาะเชื้อวัณโรคจากเสมหะที่ส่งตรวจทุกครั้ง การส่งเสมหะสองครั้งเพียงพอในการวินิจฉัยวัณโรคปอดได้ในผู้ป่วยเกือบทั้งหมด*