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Original Article

Incremental yield of serial sputum examinations in the diagnosis of pulmonary tuberculosis in Taiwan: Findings of a pragmatic trial

Chen-Yuan Chiang [a,b,c,*], Chih-Bin Lin [d,e], Shun-Tien Chien [f], Cheng-Hui Wang [g,h], Yi-Wen Huang [i,j], Wei-Chang Huang [k,l,m,n], Ming-Chih Yu [a,b,o], Jen-Jyh Lee [d], Hans L. Rieder [p]

[a] *Division of Pulmonary Medicine, Department of Internal Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*
[b] *Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan*
[c] *International Union Against Tuberculosis and Lung Disease, Paris, France*
[d] *Division of Chest Medicine, Department of Internal Medicine, Hualien Tzu Chi Hospital, Hualien, Taiwan*
[e] *School of Medicine, Tzu Chi University, Hualien, Taiwan*
[f] *Chest Hospital, Ministry of Health and Welfare, Tainan, Taiwan*
[g] *Department of Laboratory Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*
[h] *School of Medical Laboratory Science and Biotechnology, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan*
[i] *Division of Chest Medicine, Department of Internal Medicine, Changhua Hospital, Ministry of Health and Welfare, Changhua, Taiwan*
[j] *Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan*
[k] *Department of Post-Baccalaureate Medicine, College of Medicine, National Chung Hsing University, Taichung, Taiwan*
[l] *School of Medicine, Chung Shan Medical University, Taichung, Taiwan*
[m] *Division of Chest Medicine, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan*
[n] *Mycobacterial Center, Taichung Veterans General Hospital, Taichung, Taiwan*
[o] *School of Respiratory Therapy, College of Medicine, Taipei Medical University, Taipei, Taiwan*
[p] *Tuberculosis Consultant Services, Kirchlindach, Switzerland*

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* Corresponding author. 111 Hsin-Long Road, Section 3, Taipei, 116, Taiwan.
E-mail address: cychiang@theunion.org (C.-Y. Chiang).

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KEYWORDS
Smear;
Nucleic acid amplification test;
Culture;
Incremental yield;
Tuberculosis

Abstract *Background:* Presumptive tuberculosis (TB) cases commonly had two to three sputum examinations in Taiwan. The incremental yield of serial sputum examinations has not been assessed before.
Methods: In a pragmatic trial, presumptive TB patients with a frontline nucleic acid amplification test (NAAT) were classified as group A. Those without a frontline NAAT were randomized into group B frontline NAAT as intervention, and group C usual care. We investigated expected incremental yields and the number of examinations required for detection of one additional TB case from each serial sputum smear and culture.
Results: Of 6835 presumptive TB cases, 395 (5.8%) were smear positive for acid-fast bacilli, and 195 (2.8%) culture positive for *M tuberculosis*. The expected incremental yield from a third smear was 3.5% and examination of 1712 (95% credibility interval 586—4706) third smears was required to detected one additional TB case. Sensitivity of one smear with an NAAT in group B was 46.8% (95% confidence interval 32.1%—61.9%), and that of two smears in Group C 40.0% (95% confidence interval 25.7%—55.7%). The expected incremental yield from a third culture was 8.4%, and the number of third cultures required to detect one additional TB case was 394 (95% credibility interval 231—670).
Conclusions: The incremental yield of the third sputum smear was negligible. It may be reasonable to perform an NAAT, smear and culture on the first specimen and culture alone on the second. The utility of the third serial culture for the detection of additional TB case is debatable.
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Introduction

Tuberculosis (TB) is an important public health problem globally. Sputum examinations are essential for the diagnosis of pulmonary TB. Examinations of three sputum specimens produced by presumptive TB cases have been recommended.[1,2] However, studies have revealed that the yield of additional positive results detected by the third specimen could be too small to justify the workload.[3—5] A systematic review reported that the average proportion of sputum smear positive results detected by the first specimen was 85.8%.[6] The average incremental yield of the second examination was 11.9%, and that of the third specimen was 4.1% in studies using processed smears and 2.3% in studies using direct smear microscopy.[6] However, incremental yields in serial sputum examinations may not reflect laboratory workload required for detection of one additional TB case, which may vary greatly depending on the prevalence of TB among examinees.[7—10]

To estimate the workload, we had proposed a method to quantify the number of examinations required to detect one additional positive result in serial sputum examinations.[10] Seven international experts were requested to provide their opinion on the maximum number of sputum smear examinations that may justify the requirement of the third serial smear; and the proposed value ranged from 40 to 200,[10] under the assumption that the reasonable workload of a microscopist was not more than 25 smears per working day.

The number of TB cases notified in Taiwan was 16,472 (72.5 per 100,000 population) in 2005, decreasing to 7062 (30.1 per 100,000 population) in 2021. Taiwan TB guidelines recommended to perform smear and culture examinations on two to three sputum specimens, as well as a nucleic acid amplification test (NAAT) as a frontline test on the first specimen.[11] However, NAAT has been under-used in the diagnosis of pulmonary TB, likely due to the reimbursement policy of national health insurance,[12,13] and the incremental yield of serial sputum examinations in Taiwan has not been properly evaluated before. We thus conducted a pragmatic trial on the use of an NAAT as a frontline test for the diagnosis of pulmonary TB. We assessed the incremental yield of serial sputum examinations and quantified the number of sputum examinations required to detect one additional TB case in the pragmatic trial. We report findings of the assessment.

Methods

The pragmatic trial on the use of an NAAT in the diagnosis of TB among presumptive TB cases aged 20 years or more was conducted in five hospitals in Taiwan. The trial did not intervene with routine practice of clinicians in requesting an NAAT as a frontline test, together with smear and culture examinations for patients suspected of having pulmonary TB. These patients were classified as group A (frontline NAAT by clinician). Those who had no NAAT were randomized in a 1:1 ratio into group B for whom an NAAT as a frontline test was performed as intervention, and group C (usual care) for whom no frontline NAAT was performed. The trial was approved by the Joint Institutional Review Board of Taipei Medical University (N202002038) and registered at ClinicalTrials.gov (ID: NCT04433195). It has been specified in the trial design that incremental yield of serial sputum examinations will be evaluated.

We adapted the method proposed earlier[10] to assess the incremental yield of serial sputum examinations and the number of specimens required to detect one additional TB

case with each serial examination. Details of the calculation are provided in supplement 1.

We included all trial participants in calculating the incremental yield of sputum smear examinations. Because a positive smear could be due to either *M tuberculosis* or a nontuberculous mycobacterium (NTM), we analyzed patterns of serial smears for positive smear results (due to TB or NTM), and positive smears that were culture positive for *M tuberculosis*. As some patients may have missed second and third examinations, we calculated the expected number and proportion of a positive smear result and that of a positive smear result that was culture positive for *M tuberculosis* for each serial examination, taking into account the potential positive results of missing examinations (supplement 1). We then calculated the expected incremental yield from each serial smear for a positive smear result and a positive smear that was culture positive for *M tuberculosis*, and the expected number of smear examinations required to identify one positive smear result and one additional TB case with each serial sputum smear examination.

Participants of Group A and Group B had an NAAT, a smear and a culture on the first specimen, followed by the second and third sputum smear and culture. We analyzed patterns of positive results of an NAAT and serial smear examinations, and positive results that were culture positive for *M tuberculosis*. We calculated the expected number and proportion with a positive result (due to TB or NTM) and that of a positive result that was culture positive for *M tuberculosis*, the expected incremental yield from serial examinations, and the number of examinations required to identify one positive result and one additional TB case with each serial sputum examination.

We included all trial participants in calculating the incremental yield of serial sputum cultures. Because a positive culture could be either *M tuberculosis* or NTM, we analyzed patterns of serial culture examinations for both culture positive for mycobacteria (either *M tuberculosis* or NTM) and culture positive for *M tuberculosis*. We calculated the expected number and proportion culture positive for mycobacteria and that of culture positive for *M tuberculosis*. We then calculated the potential incremental yield from serial cultures, and the number of cultures required to identify one additional positive culture (either *M tuberculosis* or NTM) and one additional TB case with each serial sputum culture examination.

We computed cumulative sensitivity and Clopper-Pearson 95% confidence interval (CI) of serial sputum examination for smear, the NAAT and culture in the detection of *M tuberculosis*.

To calculate credibility intervals for the number of smears and cultures required to identify one additional TB case, a Bayesian analysis using the Markov Chain Monte Carlo approach was done with WinBUGS (Imperial College & Medical Research Council, UK, Version 1.4.3, 2007). We set the burn-in period to 5000 and the sample size to 100,000 iterations.

We also computed the expected incremental yield from each serial sputum examination for a positive result for NTM, and the expected number of smear and culture examinations required to identify one additional NTM case with each serial sputum examination.

Results

A total of 6835 presumptive TB cases were enrolled in the pragmatic trial. 1116 (16.3%) had a frontline NAAT requested by clinicians (Group A), 2838 (41.5%) were randomized into Group B and 2881 (42.2%) Group C. Of the 6835 cases, 395 (5.8%) were smear positive, 6429 (94.1%) smear negative, and 11 (0.1%) had no smear examination done; 195 (2.8%) were culture positive for *M tuberculosis*, 1085 (16.0%) culture positive for NTM, 5544 (81.1%) culture negative, and 11 (0.1%) had no culture result. Of the 1116 cases of Group A, 249 (22.3%) were smear positive; 89 (8.0%) were NAAT positive; 103 (9.2%) were culture positive for *M tuberculosis* and 260 (23.3%) culture positive for NTM. Of the 2838 cases of Group B, 79 (2.8%) were smear positive, 34 (1.2%) were NAAT positive; 47 (1.6%) were culture positive for *M tuberculosis* and 408 (14.4%) were culture positive for NTM. Of the 2881 cases of Group C, 67 (2.3%) were smear positive, 45 (1.5%) were culture positive for *M tuberculosis* and 417 (14.5%) culture positive for NTM.

Of the 6824 presumptive TB patients with sputum smear examinations, 4538 (66.5%) had three smears examined, 836 (12.3%) had two smears examined, and 1450 (21.3%) had one smear examined. Of the 395 patients with positive smear results, 109 (27.6%) had culture-confirmed *M tuberculosis*. The expected incremental yield of the third smear for any additional positive smear result was 11.8%, and that for additional positive smear that was culture positive for *M tuberculosis* was 3.5% (Table 1). The expected number of third smears that were required for detection of one additional positive smear that was culture positive for *M tuberculosis* was 1712 (95% credibility interval 586—4706) (Fig. 1). Among all presumptive TB cases, sensitivity of the first smear in the detection of *M tuberculosis* was 44.6% (95% CI 37.5%—51.9%), and that of two smears (first and second) 54.4% (95% CI 47.1%—61.5%), and that of three smears 55.9% (95% CI 48.6%—63.0%).

Of the 3943 presumptive TB cases of Group A and Group B who had an NAAT and smear on the first specimen and a smear on the second and third specimen, 356 (9.0%) had positive results, among which 109 (30.6%) were culture positive for *M tuberculosis*. The potential incremental yield of the third smear for an additional positive smear result that was culture positive for *M tuberculosis* was 1.2%. The expected number of third smears that was required for detection of one additional positive case that was culture positive for *M tuberculosis* was 3031 (Table 2).

Of the 1116 patients of Group A, 81 (7.3%) had a positive NAAT or smear results that were culture positive for *M tuberculosis*. The third smear did not detect any additional positive result that was culture positive for *M tuberculosis* (Table 2). Sensitivity of the first smear examination in the detection of positive cultures of *M tuberculosis* was 64.1% (95% CI 54.0%—73.3%), and that of an NAAT and smear on the first specimen was 76.7% (95% CI 67.3%—84.5%), and that of two examinations (NAAT on the first and smear on the first and second specimen) was 78.6% (95% CI 69.5%—86.1%). The third smear did not detect any additional TB case.

Of the 2838 patients of Group B, 18 (0.6%) had positive smears that were culture positive for *M tuberculosis* (Table 1). An NAAT on the first specimen added 10 positive results

Table 1 Results of sputum smear examinations among presumptive tuberculosis (TB): patterns among serial smear results, observed proportion smear positive and smear positive that were culture positive for *M tuberculosis*, expected number and proportion smear positive and smear positive that were culture positive for *M tuberculosis*, potential incremental yield from serial smears for a positive smear result and a positive smear that were culture positive for *M tuberculosis*, and number of smears required to identify one positive smear result and one additional TB case with each serial sputum smear examination.

		Smear positive[a]	Smear positive for TB[b]			
			All	Group A	Group B	Group C
Ad	Total (Px + NPx + NNP + NNN + NN9+N99)	6824	6824	1105	2838	2881
Px	Positive on first	267	87	66	10	11
NPx	Negative on first, positive on second	90	19	5	7	7
NNP	Negative on first and second, positive on third	38	3	2	1	0
NNN	Negative on three	4215	4467	883	1803	1781
NN9	Negative on first and second, missing third	793	831	103	345	383
N99	Negative on first, missing second and third	1421	1417	46	672	699
Od	Observed number positive (Px + NPx + NNP)	395	109	73	18	18
Sd	Observed proportion positive (Od/Ad)	5.79%	1.60%	6.61%	0.63%	0.62%
Sd1	Proportion positive on first (Px/Ad)	3.91%	1.27%	5.97%	0.35%	0.38%
Sd2	Proportion positive on second (NPx/Ad)	1.32%	0.28%	0.45%	0.25%	0.24%
Sd3	Proportion positive on third (NNP/Ad)	0.56%	0.04%	0.18%	0.04%	0.00%
Md2	Positive missed on second (Sd2*N99)	19	4	0	2	2
Md3	Positive missed on third [Sd3*(NN9+(1-Sd2)* N99)]	12	1	0	0	0
Md	Positive missed total (Md2+Md3)	31	5	0	2	2
Ed	Number positive observed and missed (Od + Md)	426	114	73	20	20
Rd	Expected proportion positive (Ed/Ad)	6.24%	1.67%	6.65%	0.71%	0.68%
Fd1	Potential incremental yield first (Px/Ed)	62.68%	76.36%	89.82%	49.96%	55.84%
Fd2	Potential incremental yield second [(Md2+NPx)/Ed]	25.53%	20.14%	7.09%	43.25%	44.16%
Fd3	Potential incremental yield third [(Md3+NNP)/ Ed]	11.79%	3.50%	3.09%	6.78%	0.00%
Rd1	Expected proportion positive on first (Rd*Fd1)	3.91%	1.27%	5.97%	0.35%	0.38%
Rd2	Expected proportion positive on second (Rd*Fd2)	1.59%	0.34%	0.47%	0.31%	0.30%
Rd3	Expected proportion positive on third (Rd*Fd3)	0.74%	0.06%	0.21%	0.05%	0.00%
N1	Number examined per case first (1/Rd1)	26	78	17	284	262
N2	Number examined per case second (1/Rd2)	63	297	212	328	331
N3	Number examined per case third (1/Rd3)	136	1712	487	2090	NC[c]

[a] Smear positive for mycobacteria.
[b] Smear positive specimens that were culture positive for *M tuberculosis*. A positive smear result was re-classified as a negative result if the specimen was culture positive for nontuberculous mycobacteria.
[c] NC: Not calculable because Rd3 = 0.

that were culture positive for *M tuberculosis*, compared to first smear alone (Table 2). Sensitivity of the first smear in the detection of positive cultures for *M tuberculosis* was 21.3% (95% CI 10.7%—35.7%), that of an NAAT and smear on the first specimen was 46.8% (95% CI 32.1%—61.9%), that of two examinations (NAAT on the first and smear on the first and second specimen) was 57.5% (95% CI 42.2%—71.7%), and that of all three examinations was 59.6% (95% CI 44.3%—73.6%).

Among presumptive TB patients of Group A and Group B of the five participating hospitals, the third smear did not detect any additional TB case in four hospitals (Supplement Table 1).

Among Group C for whom no frontline NAAT was performed, 18 (0.6%) had positive smears that were culture positive for *M tuberculosis* (Table 1). Sensitivity of the first smear in the detection of 45 positive cultures for *M tuberculosis* was 24.4% (95% CI 12.9%—39.5%), and that of two smears 40.0% (95% CI 25.7%—55.7%). The third smear did not detect any additional TB case.

Of the 6824 patients with sputum culture examinations, 4538 (66.5%) had three cultures examined, 836 (12.3%) two cultures examined, and 1450 (21.3%) one culture examined (Table 3). Of the 1280 patients with a positive sputum culture, in 195 (15.2%) the culture was positive for *M tuberculosis*. The potential incremental yield of the third culture for an additional positive culture for mycobacteria was 14.9%, and that for an additional positive culture for *M tuberculosis* was 8.4%. The expected number of the third culture required for the detection of one additional positive culture for *M tuberculosis* was 394 (95% credibility interval 231—670) (Fig. 2). The expected number of third cultures

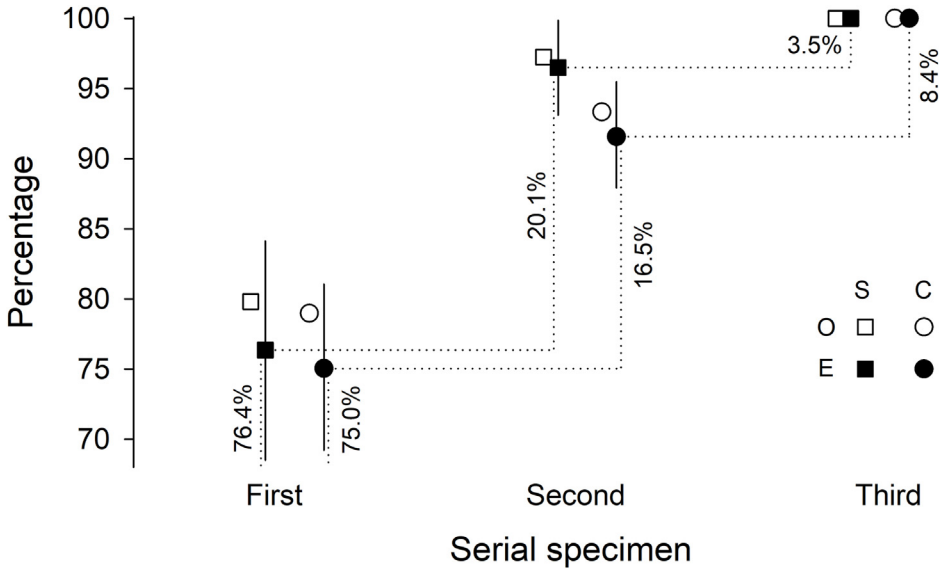

Figure 1. Incremental yield in the identification of additional tuberculosis cases and the cumulative proportion positive on serial sputum examinations, by smear (squares) and culture (circles). S, smear; C, culture; O, observed proportion positive; E, expected proportion positive. Expected incremental yield (dotted line), observed cumulative proportion positive (hollow symbols), expected cumulative proportion positive (filled symbols), 95% confidence interval of expected cumulative proportion positive (vertical straight line) of serial sputum examinations.

required to detect one additional TB case per hospital ranged from 272 to 1278 (Supplement Table 2).

The expected number of the third smear and culture required for the detection of one additional NTM case was 347 and 34, respectively (Supplement Table 3).

Discussion

In this pragmatic trial, the majority of positive cultures were NTM and the proportion of patients with a positive culture for *M tuberculosis* was relatively low. Performing an NAAT and smear examination on the first specimen had a higher proportion of positive results that were culture positive for *M tuberculosis* as compared with sputum smear alone. Sputum culture helped detect a fraction of TB cases missed by smear and NAAT. Examinations of a large number of third smears were required to detect one additional TB case, and the number of third cultures examined to detect one additional positive culture for *M tuberculosis* was also remarkably high.

Incremental yields of serial sputum smear examinations in the diagnosis of pulmonary TB have been investigated, but the related laboratory workload for the detection of one additional TB case in serial sputum examinations have rarely been assessed.[6] We have previously reported that the incremental yield from the third smear examination was 2.5% in Benin, 0.7% in Malawi, 3.9% in Moldova, 7.2% in Nicaragua, 3.0% in Senegal, and 2.7% in Uganda, and that the number of the third examination required to detect one additional positive smear was 123 in Benin, 796 in Malawi, 260 in Nicaragua, 273 in Moldova, 177 in Senegal and 175 in Uganda.[9,10] Sputum culture was not routinely performed in these countries. Therefore, it was not possible to assess whether a positive sputum smear was due to *M tuberculosis* or NTM. Incremental yields of serial sputum culture examinations have rarely been investigated. Ismail et al. reported that the incremental yield from the third culture examination was 8.3% among smear-negative HIV-infected presumptive case in South Africa.[14] The incremental yield from the third culture examination in Portugal was 1.3% in one study and 3.5% in another.[7,15] These three studies did not investigate the number of the third culture examination required to detect one additional TB case. Ssengooba et al. reported that in Uganda of the 170 smear negative presumptive TB case, 62 (36.5%) were culture positive for *M tuberculosis*.[8] The incremental yield from the third culture examination was 7.3% and the number of the third culture examination required to detect one additional TB case was 36.[8]

In our study, the proportion of patients with a culture positive for *M tuberculosis* in Group A was higher than that in Group B and Group C. Among presumptive TB cases of Group A, the third smear did not detect any additional positive result that was culture positive for *M tuberculosis*, thus cannot be justified. The number of third cultures required for detection of one additional culture positive TB case was relatively high. The utility of the third culture in the diagnosis of TB will require further consideration. Dropping the third culture may reduce the burden of transportation for the majority of presumptive TB patients, and the potential biosafety hazards of laboratory staff in collecting and processing sputum specimens. For patients who are still suspected of having pulmonary TB, another set of two specimens may be requested. The number of third smears required for detection of one additional NTM case remained substantial but that for the third cultures was relatively low, indicating that the detection strategy for NTM may require further assessment.

Those who did not have an NAAT as a frontline test requested by the clinician had a relatively low probability of having a positive culture for *M tuberculosis*. Compared with

Table 2 Nucleic acid amplification test (NAAT) on the first specimen and serial smear examinations among presumptive tuberculosis (TB): patterns among NAAT and serial smear results, observed proportion positive, expected number and proportion positive, potential incremental yield from serial examinations, and number of examinations required to identify one additional positive result and one additional TB case with each serial sputum examination, Group A and Group B.

		Any positive[a]	NAAT or smear positive for TB[b]		
			All	Group A	Group B
Ad	Total (Px + NPx + NNP + NNN + NN9+N99)	3943	3943	1105	2838
Px	Positive on first NAA and/or smear	268	101	79	22
NPx	Negative on first test, positive on second smear	60	7	2	5
NNP	Negative on first and second test, positive on third smear	28	1	0	1
NNN	Negative on three	2455	2647	854	1793
NN9	Negative on first and second, missing third	415	448	103	345
N99	Negative on first, missing second and third	717	739	67	672
Od	Observed number positive (Px + NPx + NNP)	356	109	81	28
Sd	Observed proportion positive (Od/Ad)	9.03%	2.76%	7.33%	0.99%
Sd1	Proportion positive on first (Px/Ad)	6.80%	2.56%	7.15%	0.78%
Sd2	Proportion positive on second (NPx/Ad)	1.52%	0.18%	0.18%	0.18%
Sd3	Proportion positive on third (NNP/Ad)	0.71%	0.03%	0.00%	0.04%
Md2	Positive missed on second (Sd2*N99)	11	1.3	0.1	1.2
Md3	Positive missed on third [Sd3*(NN9+(1-Sd2)* N99)]	8	0.3	0.0	0.4
Md	Positive missed total (Md2+Md3)	19	1.6	0.1	1.5
Ed	Number positive observed and missed (Od + Md)	375	110.6	81.1	29.5
Rd	Expected proportion positive (Ed/Ad)	9.51%	2.81%	7.34%	1.04%
Fd1	Potential incremental yield first (Px/Ed)	71.49%	91.31%	97.39%	74.47%
Fd2	Potential incremental yield second [(Md2+NPx)/Ed]	18.92%	7.52%	2.61%	20.94%
Fd3	Potential incremental yield third [(Md3+NNP)/Ed]	9.59%	1.18%	0.00%	4.60%
Rd1	Expected proportion positive on first (Rd*Fd1)	6.80%	2.56%	7.15%	0.78%
Rd2	Expected proportion positive on second (Rd*Fd2)	1.80%	0.21%	0.19%	0.22%
Rd3	Expected proportion positive on third (Rd*Fd3)	0.91%	0.03%	0.00%	0.05%
N1	Number examined per case first (1/Rd1)	15	39	14	129
N2	Number examined per case second (1/Rd2)	56	474	521	459
N3	Number examined per case third (1/Rd3)	110	3031	NC[c]	2090

[a] Positive for mycobacteria.
[b] NAAT positive or smear positive specimens that were culture positive for *M tuberculosis*. A positive smear and NAAT result was re-classified as a negative result if the specimen was culture positive for nontuberculous mycobacteria.
[c] NC: Not calculable because Rd3 = 0.

smear alone, adding an NAAT as a frontline test on the first specimen in Group B increased the sensitivity of the first sputum examination in the detection of *M tuberculosis*. Sensitivity of the first smear with a frontline NAAT in group B was higher than that of two smears in group C. We used Xpert MTB/RIF as the NAAT for Group B.[16] Whether instead Xpert MTB/RIF Ultra would further increase the sensitivity of the first examination needs additional assessment.[17] In group B, the number of third smears required to detect one additional positive result that was culture positive for *M tuberculosis* was 2090, equivalent to 83 working days (assuming 25 smears/day) for a laboratory person. The third smear did not detect any additional TB case in Group C. Therefore, routinely performing the third smear in patients with a low probability of TB in Taiwan cannot be justified.[18] The number of third cultures required to detect one additional TB case in Group B and Group C was relatively high. The utility of the third sputum culture in the diagnosis of pulmonary TB among patients with presumptive TB with a low pretest probability is questionable.

Among presumptive TB cases with an NAAT as a frontline test, the number of second smears required to detect one additional positive result that was culture positive for *M tuberculosis* was also considerably high. Since the small incremental yield of the second smear in the detection of additional TB case will be captured by the second culture, it might be reasonable to perform culture alone on the second specimen without smear in those who had NAAT as a frontline test on the first specimen.

A strength of the study was that this was a pragmatic trial, thus reflecting the real-world condition of clinical practice in the diagnosis of pulmonary TB in Taiwan. Furthermore, the number of presumptive TB cases was relatively large, helping to ensure the precision of findings. Moreover, we analyzed positive smears and cultures separately for any mycobacterium and only *M tuberculosis*, thus avoiding the confusion that may be caused by NTM. A limitation of the study was that it was conducted at five hospitals in Taiwan, and findings may thus not be generalizable to other facilities.

Table 3 Results of serial sputum culture examinations that were culture positive for mycobacteria and for *M tuberculosis* among presumptive tuberculosis (TB): patterns among serial culture results, observed proportion positive, expected number and proportion positive, expected incremental yield from serial cultures, and number of cultures required to identify one positive culture result and one additional TB case with each serial sputum culture examination.

		Positive culture[a]	Culture positive for *M tuberculosis*			
			All	Group A	Group B	Group C
Ad	Total (Px + NPx + NNP + NNN + NN9+N99)	6824	6824	1105	2838	2881
Px	Positive on first	814	154	89	33	32
NPx	Negative on first, positive on second	306	28	9	8	11
NNP	Negative on first and second, positive on third	160	13	5	6	2
NNN	Negative on three	3504	4358	833	1775	1750
NN9	Negative on first and second, missing third	708	828	102	344	382
N99	Negative on first, missing second and third	1332	1443	67	672	704
Od	Observed number positive (Px + NPx + NNP)	1280	195	103	47	45
Sd	Observed proportion positive (Od/Ad)	18.76%	2.86%	9.32%	1.66%	1.56%
Sd1	Proportion positive on first (Px/Ad)	11.93%	2.26%	8.05%	1.16%	1.11%
Sd2	Proportion positive on second (NPx/Ad)	4.48%	0.41%	0.81%	0.28%	0.38%
Sd3	Proportion positive on third (NNP/Ad)	2.34%	0.19%	0.45%	0.21%	0.07%
Md2	Positive missed on second (Sd2*N99)	59.8	5.9	1	2	3
Md3	Positive missed on third [Sd3*(NN9+(1-Sd2)* N99)]	46.5	4.3	1	2	1
Md	Positive missed total (Md2+Md3)	106.2	10.2	1	4	3
Ed	Number positive observed and missed (Od + Md)	1386.2	205.2	104	51	48
Rd	Expected proportion positive (Ed/Ad)	20.31%	3.01%	9.44%	1.80%	1.68%
Fd1	Potential incremental yield first (Px/Ed)	58.72%	75.03%	85.32%	64.66%	66.06%
Fd2	Potential incremental yield second [(Md2+NPx)/Ed]	26.39%	16.53%	9.15%	19.39%	28.26%
Fd3	Potential incremental yield third [(Md3+NNP)/Ed]	14.89%	8.44%	5.52%	15.96%	5.68%
Rd1	Expected proportion positive on first (Rd*Fd1)	11.93%	2.26%	8.05%	1.16%	1.11%
Rd2	Expected proportion positive on second (Rd*Fd2)	5.36%	0.50%	0.86%	0.35%	0.48%
Rd3	Expected proportion positive on third (Rd*Fd3)	3.03%	0.25%	0.52%	0.29%	0.10%
N1	Number examined per case first (1/Rd1)	8	44	12	86	90
N2	Number examined per case second (1/Rd2)	19	201	116	287	210
N3	Number examined per case third (1/Rd3)	33	394	192	348	1047

[a] Culture positive for either *M tuberculosis* or nontuberculous mycobacteria.

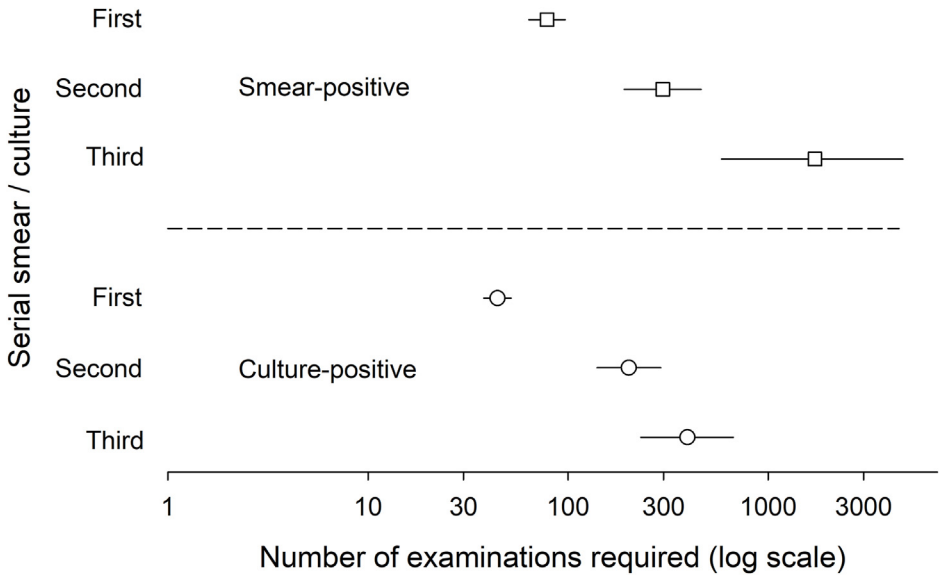

Figure 2. Number of examinations required for successive serial sputum examinations to identify one additional tuberculosis case, by smear (squares) and culture (circles). Horizontal straight lines show 95% credibility intervals for the number of examinations required to identify one additional tuberculosis case with serial diagnostic examinations. Horizontal dashed line separate smear and culture.

In conclusion, the incremental yield of third sputum smear examination in the detection of additional TB case was negligible. It may be reasonable to perform an NAAT, smear and culture on the first specimen and culture alone on the second. The utility of the third serial culture in the detection of additional TB case in Taiwan is debatable. If two sputum specimens are examined, those which are negative on both examinations, yet TB still cannot be excluded may submit another set of two sputum specimens for examinations.

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Declaration of competing interest

None declared.

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We used the WinBUGS script that Beat Neuenschwander had written for our first article to calculate credibility intervals, modifying it for our current requirements.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2023.09.006.