

Usefulness of the polymerase chain reaction dot-blot assay, used with Ziehl-Neelsen staining, for the rapid and convenient diagnosis of pulmonary tuberculosis in human immunodeficiency virus-seropositive and -seronegative individuals

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Abstract

There are scarce data regarding the value of molecular tests, when used in parallel with classical tools, for the diagnosis of tuberculosis (TB) under field conditions, especially in regions with a high burden of TB-human immunodeficiency virus (HIV) co-infection. We evaluated the usefulness of the polymerase chain reaction dot-blot assay (PCR) used in parallel with Ziehl-Neelsen staining (ZN) for pulmonary tuberculosis (PTB) diagnosis, in a TB-HIV reference hospital. All sputum samples from 277 patients were tested by ZN, culture, and PCR. Performances were assessed individually, in parallel, for HIV status, history of anti-TB treatment, and in different simulated TB prevalence rates. Overall, the PTB prevalence was 46% (128/277); in HIV-seropositive (HIV⁺) individuals, PTB prevalence was 54% (40/74); the ZN technique had a lower sensitivity (SE) in the HIV⁺ group than in the HIV-seronegative (HIV⁻) group (43% vs. 68%; Fisher test, $P < 0.05$); and the SE of PCR was not affected by HIV status (Fisher test; $P = 0.46$). ZN, in parallel with PCR, presented the following results:

i) among all PTB suspects, SE of 90%, specificity (SP) of 84%, likelihood ratio (LR)⁺ of 5.65 and LR⁻ of 0.12; ii) in HIV⁻ subjects: SE of 92%, LR⁻ of 0.10; iii) in not previously treated cases: SE of 90%, LR⁻ of 0.11; iv) in TB, prevalence rates of 5-20%; negative predictive values (NPV) of 98-99%. ZN used in parallel with PCR showed an improvement in SE, LR⁻, and NPV, and may offer a novel approach in ruling out PTB cases, especially in not previously treated HIV⁻ individuals, attended in hospitals in developing nations.

Introduction

Tuberculosis (TB) is one of the most important health problems in the world, with 1.8 million deaths reported each year.¹ Direct smear examination with Ziehl-Neelsen (ZN) staining for the diagnosis of pulmonary tuberculosis (PTB), as employed in most low-income countries, is cheap and easy to use, but its low sensitivity is a major drawback.² In Brazil, ZN is the recommended method both for TB diagnosis and treatment control, and sputum culture in solid medium is only indicated in PTB-suspect cases, such as those with: i) ZN-negative results; ii) paucibacillary and extrapulmonary specimens; iii) therapeutic failure with suspicion of drug resistance; and iv) individuals infected by HIV.³ Rapid TB diagnosis has become crucial, especially for diagnosis involving clinical specimens from subjects with atypical presentation, where direct microscopy presents low sensitivity and culture can delay diagnosis by three to six weeks.¹

Important advances in molecular techniques, which rapidly identify mycobacterial DNA in sputa, may overcome these obstacles.² In developing countries, in-house polymerase chain reaction assays (PCR) for the amplification of *Mycobacterium tuberculosis* (MTB) DNA, using the IS6110 insertion as a PCR target, could be a quick diagnostic test for TB and offers the potential of a sensitive, specific, and rapid diagnostic tool for ruling out pulmonary tuberculosis (PTB). However, PCR methods in respiratory specimens present some caveats: i) reaction inhibitors; ii) lower sensitivity in paucibacillary specimens; and iii) high costs.

The majority of previous studies have evaluated in-house and automated PCR and reported PCR sensitivities ranging from 77% to 95% and PCR specificities of 95% in smear-positive specimens, using culture as the gold standard and clinical criteria only to evaluate the inconsistent results.⁴ Moreover, the PCR tests were evaluated separately, in contrast to clinical practice where associated tests are required for diagnosis. More recently, the evaluation of the usefulness of PCR, in parallel with the classical diagnostic techniques for rapid diagnosis

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of TB, has been considered a novel approach.⁵⁻⁷ In order to compare the performance of the use of a molecular test (PCR dot-blot assay) or culture in parallel with ZN for the diagnosis of PTB, we conducted a prospective study in a TB-HIV reference hospital, located in Porto Alegre City in the south of Brazil where, in 2004, 1432 TB cases were reported, 420 of them diagnosed in hospitals and 51% being HIV-infected patients.⁸

Materials and Methods

Consecutive patients, adults suspected of having PTB and referred to the TB and HIV Reference Center, Parthenon Reference Hospital in Porto Alegre City, capital of Rio Grande do Sul, State of Brazil, were studied prospectively. PTB suspects were referred from community health care units to have their respiratory specimens cultured for mycobacteria, according to Brazilian National Guidelines.³ Eligible patients were those who reported more than three weeks of coughing; ineligible patients were those receiving anti-TB treatment. Patients were excluded from the study if any of the following conditions were met: i) the culture was contaminated; ii) when expectorated sputum was not obtained; iii) laboratory or clinical data did not fulfill the PTB definition; and iv) written informed consent was

not obtained from the study participant. All clinical samples were sent to the Laboratory of the State of RS, State Foundation for Research in Health, Porto Alegre, RS, Brazil (FEPPS, Lacer, RS) for laboratory analysis. All clinical specimens were processed using the acetyl-cysteine method. Ziehl-Neelsen staining (ZN) and culture (Lowenstein Jensen solid medium) were performed following routine procedures. Positive cultures were submitted to standard identification procedures for differentiation of the MTB complex from atypical mycobacteria.⁹ The PCR dot-blot assay was performed as previously described.¹⁰ Briefly, using the IS6110 insertion element as a target for PCR, PCR products were transferred to a nylon membrane, and hybridization was performed with a specific biotinylated probe. The detection of hybridization was performed using conjugated streptavidin-alkaline phosphatase. A positive reaction was obtained by adding 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro-blue tetrazolium (NBT). Positive and negative controls were included for each PCR set. In order to detect specimen inhibitors in negative results, a tube of PCR mix for each specimen was spiked with the purified DNA target.¹⁰ All PCR tests with discrepancies in the results were assayed in duplicate.

Suspects of PTB, after signing their written informed consent, underwent a validated questionnaire with questions regarding demographic variables and clinical history (e.g. smoking, alcohol abuse, HIV infection/AIDS).¹¹ Chest radiographs and physical examinations were performed by a respiratory specialist using a standardized form. Respiratory specialists were blinded as to the results of culture and PCR, and laboratory technicians were blinded as regards the chest radiographs and clinical predictors. HIV-testing by ELISA was performed using Western blot as a confirmatory test. The gold standard was the combination of a positive culture with a clinical definition of PTB.¹⁰ Clinical and final diagnosis of confirmed PTB cases were defined as those with a positive culture for MTB in the respiratory specimen; presumptive PTB as those showing clinical improvement after six months of anti-TB treatment, as judged by three different chest physicians not involved in this study in a blinded manner.¹² Non-PTB was considered when patients had a negative acid-fast smear and MTB culture, and did not present clinical and chest radiographic changes after six months of follow-up.

Test performances were calculated using specific formulae as a function of sensitivity (SE) and specificity (SP) of PCR used in parallel with the ZN smear examination:

$$SE_{ZN} + SE_{PCR} - (SE_{ZN} \times SE_{PCR});$$

used for parallel tests, predictive values (PV) for different simulated statistical prevalence rates, and likelihood ratios (LR), according to

the literature.¹³

Although the information in a diagnostic test can be summarized using SE and SP, other parameters may be important clinically for the definition of the accuracy of a laboratory test. LRs allow the investigator to take advantage of all information in a test. For each test result, the likelihood ratio is the ratio of the likelihood of that result in someone with the disease to the likelihood of that result in someone who does not have the same disease. The LR for a positive test is:

$$SE / (1-SP),$$

and the LR for a negative test is

$$(1-SE/SP).$$

The higher the LR, the better the test result for ruling in a diagnosis; a LR of greater than 100 is very high (and very unusual among tests). On the other hand, the lower a LR (the closer it is to 0), the better the test result is for ruling out the disease.

The positive PV (PPV) is the proportion of true positives in all positive results, and shows the probability that one patient with a positive test has the disease. The negative PV (NPV) is the proportion of true negatives in all negative results and shows the probability that one

patient with a negative test does not have the disease.

Ethics

Written informed consent was obtained from all patients, and HIV was tested by ELISA, using the Western blot as a confirmatory test. This study was approved by the Institutional Review Boards of FEPPS (n. 01/2002).

Results

From May 2003 to May 2004, 277 patients with suspected PTB were recruited at the Parthenon Reference Hospital, a reference center for TB and HIV, in Porto Alegre. No atypical mycobacteria were isolated from clinical samples during the study period. Overall, PTB prevalence was 46.0% (128/277), 54.0% (40/74) among HIV-seropositive (HIV⁺) individuals, 53.7% (109/203) in those individuals not previously treated for TB, and 25.7% (19/74) in those cases with a history of anti-TB treatment.

Chest X-rays, suggestive of classical TB (any

Table 1. Performance of Ziehl-Neelsen staining, culture, and polymerase chain reaction dot-blot assays, according to the history of anti-tuberculosis treatment.

	Laboratory results and performance of methods					
	All groups N=277		Non-treated TB group N=203		Past-TB group N=74	
	TB N=128	Non-TB N=149	TB N=109	Non-TB N=94	TB N=19	Non-TB N=55
ZN						
Positive	77	1	68	0	9	1
Negative	51	148	41	94	10	54
	SE	SP	SE	SP	SE	SP
	60%	99%	62%	100%	47%	98%
	LR ⁺	LR ⁻	LR ⁺	LR ⁻	LR ⁺	LR ⁻
	60	0.40	62	0.38	24	0.54
Culture						
Positive	107	0	94	0	13	0
Negative	21	149	15	94	6	55
	SE	SP	SE	SP	SE	SP
	84%	100%	86%	100%	68%	100%
	LR ⁺	LR ⁻	LR ⁺	LR ⁻	LR ⁺	LR ⁻
	84	0.16	86	0.14	68	0.32
PCR dot-blot assay						
Positive	95	22	83	13	12	9
Negative	33	127	26	81	7	46
	SE	SP	SE	SP	SE	SP
	74%	85%	76%	87%	63%	84%
	LR ⁺	LR ⁻	LR ⁺	LR ⁻	LR ⁺	LR ⁻
	4.93	0.31	5.9	0.27	3.93	0.44
	Performance of methods used in parallel					
ZN in parallel	SE	SP	SE	SP	SE	SP
with culture	94%	99%	94%	99%	87%	99%
ZN in parallel with	SE	SP	SE	SP	SE	SP
PCR dot-blot assay	90%	84%	90%	86%	85%	83%

SE, sensitivity; LR⁺, positive likelihood ratio; SP, specificity; LR⁻, negative likelihood ratio.

parenchymal infiltrate or cavity located in the upper zone, defined as the area above the third rib posteriorly), were more frequently observed in HIV-seronegative (HIV⁻) patients than in HIV⁺ ones (67.3% vs. 32.2%; Fisher test; $P < 0.05$). Overall, PCR presented a higher SE than that of the ZN method (74% vs. 60%; χ^2 test; $P < 0.05$) and was lower than that observed with culture (74% [CI 95%: range: 71-79%] vs. 84% [CI 95%: 78-96%]; χ^2 test; $P = 0.06$) (Table 1). Culture and PCR presented LR⁺ values of 84.0 and 4.93 and LR⁻ values of 0.16 and 0.31. ZN used in parallel with culture demonstrated a SE of 94%, SP of 99%, LR⁺ of 93.6, and LR⁻ of 0.06 (Table 1). ZN used in parallel with PCR demonstrated a SE of 90%, SP of 84%, LR⁺ of 5.65, and LR⁻ of 0.12 (Table 1). A LR⁻ of close to 0 indicates a better test result for ruling out PTB. Among PTB suspects not previously treated for TB, ZN in parallel with PCR presented a SE of 90%, SP of 86%, LR⁺ of 6.52, and LR⁻ of 0.11. Additionally, ZN in parallel with culture demonstrated a SE of 94%, SP of 99%, LR⁺ of 94.4, and LR⁻ of 0.06. Among PTB suspects with a history of anti-TB treatment, ZN in parallel with PCR had a SE of 85% and SP of 83%, LR⁺ of 5.06 and LR⁻ of 0.18. Furthermore, ZN in parallel with culture had a SE of 87%, SP of 99%, LR⁺ of 87.2, and LR⁻ of 0.13.

ZN sensitivity was significantly lower among HIV⁺ subjects as compared to the HIV⁻ patients (43% [CI 95%: 41%-46%] vs. 68% [CI 95%: 64%-74%]; Fisher test; $P < 0.05$) (Tables 2 and 3). PCR sensitivity was not influenced by HIV status (72% [CI 95%: 70-77%] vs. 75% [CI 95%: 72%-81%]; Fisher test; $P = 0.46$). Among HIV⁺ subjects, PCR had a higher SE than that of ZN (72% [CI 95%: 70-77%] vs. 43% [CI 95%: 41-46%]; χ^2 ; $P < 0.05$), and similar SE to that of culture (72% [CI 95%: 70-77%] vs. 80% [CI 95%: 77-83%]; χ^2 test; $P = 0.54$) (Table 2). Culture and PCR demonstrated LR⁺ values of 80.0 and 4.80, and LR⁻ values of 0.20 and 0.33, respectively. ZN in parallel with culture had a SE of 89%, SP of 100%, LR⁺ of 88.6, and LR⁻ of 0.12. ZN in parallel with PCR had a SE of 84%, SP of 85%, LR⁺ of 5.60, and LR⁻ of 0.19. Comparing the SE and LR⁻ values among those individuals not previously treated and those treated for TB in the past, the figures were 84% and 0.18, and 86% and 0.17, respectively (Table 2). Among HIV⁻ subjects, PCR sensitivity was similar to that observed with ZN (75% [CI 95%: 72%-81%] vs. 68% [CI 95%: 64%-74%]; χ^2 test; $P = 0.36$), and with culture (75% [CI 95%: 72%-81%] vs. 85% [CI 95%: 79%-100%]; χ^2 test; $P = 0.10$) (Table 3). Culture and PCR presented LR⁺ of 85.0 and 5.0 and LR⁻ of 0.15 and 0.29, respectively. ZN in parallel with culture presented a SE of 95%, SP of 99%, LR⁺ of 47.8 and LR⁻ of 0.05. ZN in parallel with PCR had a SE of 92%, SP of 84%, LR⁺ of 5.80, and LR⁻ of 0.10. Comparing the SE and LR⁻ of ZN in parallel with PCR, among those individuals not previ-

ously treated for TB and those that used anti-TB in the past, the figures were 93% and 0.08, and 85% and 0.18, respectively (Table 3).

In our study, with a TB prevalence of 46%, the NPV and PPV of PCR observed were 81% and 79%, respectively. The use of ZN in parallel with PCR among HIV⁻ individuals showed a NPV and PPV of 93% and 83%, respectively. This strategy, among HIV⁺ individuals, had different results with a NPV and PPV of 82% and 87%, respectively. Among HIV⁺ individuals that had not previously been treated, the NPV and PPV of ZN with PCR were 88% and 82%, respectively.

Assuming different TB prevalence scenarios, the use of ZN in parallel with PCR showed similar NPVs and PPVs to those observed with ZN used in parallel with culture, among HIV⁺ and HIV⁻ patients (Table 4). ZN associated with culture presented the best performance in all scenarios. ZN associated with PCR demonstrated a different performance. In regions with a TB prevalence of 5-10%, usually in out-patient units attending individuals presenting with coughing for more than three weeks (respiratory symptoms, according to WHO), the NPV for ZN when associated with

PCR ranged from 99-100%. In health units, general hospitals, ambulatory reference centers, or TB clinics, while the TB prevalence usually ranges from 15% to 20%, NPV of this diagnostic strategy was 98-99%. In reference TB hospitals, where the TB prevalence ranges from 30% to 50%, among HIV⁻ individuals the NPV of ZN in parallel with PCR was 96%-94%, but among HIV⁺ individuals this figure was reduced to 93% and 89% (Table 4).

Discussion

The observed overall PTB prevalence of 46% and of 54% among HIV⁺ subjects confirmed the high prevalence of TB-HIV co-infection in hospital units in Brazil, as reported by the Porto Alegre City TB Control Program. This finding highlights the necessity to evaluate innovative approaches for TB diagnosis in these settings, where atypical chest X-rays and the low SE of ZN as well as the existence of paucibacillary specimens are more frequently observed in HIV⁺ patients, similar to the results described by others.⁵

Table 2. Performance of Ziehl-Neelsen staining, culture, and polymerase chain reaction dot-blot assays, according to the history of anti-tuberculosis treatment, among human immunodeficiency virus-seropositive individuals.

	Laboratory results and performance of methods					
	All groups N=74		Non-treated TB group N=47		Past-TB group N=27	
	TB N=40	Non-TB N=34	TB N=32	Non-TB N=15	TB N=8	Non-TB N=19
ZN						
Positive	17	0	14	0	3	0
Negative	23	34	18	15	5	19
	<i>SE</i>	<i>SP</i>	<i>SE</i>	<i>SP</i>	<i>SE</i>	<i>SP</i>
	43%	100%	43%	100%	37%	100%
	<i>LR</i> ⁺	<i>LR</i> ⁻	<i>LR</i> ⁺	<i>LR</i> ⁻	<i>LR</i> ⁺	<i>LR</i> ⁻
	43	0.58	43	0.57	37	0.62
Culture						
Positive	32	0	27	0	5	0
Negative	8	34	5	15	3	19
	<i>SE</i>	<i>SP</i>	<i>SE</i>	<i>SP</i>	<i>SE</i>	<i>SP</i>
	80%	100%	84%	100%	62%	100%
	<i>LR</i> ⁺	<i>LR</i> ⁻	<i>LR</i> ⁺	<i>LR</i> ⁻	<i>LR</i> ⁺	<i>LR</i> ⁻
	80	0.20	84	0.16	62	0.38
PCR dot-blot						
Positive	29	5	23	2	6	3
Negative	11	29	9	13	2	16
	<i>SE</i>	<i>SP</i>	<i>SE</i>	<i>SP</i>	<i>SE</i>	<i>SP</i>
	72%	85%	72%	87%	75%	84%
	<i>LR</i> ⁺	<i>LR</i> ⁻	<i>LR</i> ⁺	<i>LR</i> ⁻	<i>LR</i> ⁺	<i>LR</i> ⁻
	4.8	0.33	5.6	0.32	4.7	0.29
Performance of methods used in parallel						
ZN in parallel with culture	<i>SE</i>	<i>SP</i>	<i>SE</i>	<i>SP</i>	<i>SE</i>	<i>SP</i>
	89%	100%	91%	100%	78%	100%
ZN in parallel with PCR dot -blot	<i>SE</i>	<i>SP</i>	<i>SE</i>	<i>SP</i>	<i>SE</i>	<i>SP</i>
	84%	85%	84%	87%	86%	84%

SE, sensitivity; LR⁺, positive likelihood ratio; SP, specificity; LR⁻, negative likelihood ratio.

Table 3. Performance of Ziehl-Neelsen staining, culture, and polymerase chain reaction dot-blot assays, according to the history of anti-tuberculosis treatment, in human immunodeficiency virus-seronegative individuals.

	Laboratory results and performance of methods					
	All groups N=203		Non-treated TB group N=156		Past-TB group N=47	
	TB N=88	Non-TB N=115	TB N=77	Non-TB N=79	TB N=11	Non-TB N=36
ZN						
Positive	60	1	54	0	6	1
Negative	28	114	23	79	5	35
	SE	SP	SE	SP	SE	SP
	68%	99%	70%	100%	54%	97%
	LR ⁺	LR ⁻	LR ⁺	LR ⁻	LR ⁺	LR ⁻
	68	0.32	70	0.30	18	0.47
Culture						
Positive	75	0	67	0	8	0
Negative	13	115	10	79	3	36
	SE	SP	SE	SP	SE	SP
	85%	100%	87%	100%	73%	100%
	LR ⁺	LR ⁻	LR ⁺	LR ⁻	LR ⁺	LR ⁻
	85	0.15	87	0.13	73	0.27
PCR dot-blot						
Positive	66	17	60	11	6	6
Negative	22	98	17	68	5	30
	SE	SP	SE	SP	SE	SP
	75%	85%	78%	86%	54%	83%
	LR ⁺	LR ⁻	LR ⁺	LR ⁻	LR ⁺	LR ⁻
	5	0.29	5.8	0.25	3.17	0.55
	Performance of methods used in parallel					
ZN in parallel with culture	SE	SP	SE	SP	SE	SP
	95%	99%	96%	99%	91%	99%
ZN in parallel with PCR dot-blot	SE	SP	SE	SP	SE	SP
	92%	84%	93%	85%	85%	82%

SE, sensitivity; LR⁺, positive likelihood ratio; SP, specificity; LR⁻, negative likelihood ratio.**Table 4. Simulation of positive and negative predictive values of Ziehl-Neelsen staining, culture, and polymerase chain reaction dot-blot assays, according to different tuberculosis prevalence rates.**

Simulated prevalence rates	Subjects with HIV N=74		Subjects without HIV N=203	
	TB N=40	TB N=34	TB N=88	TB N=115
5%	PPV	NPV	PPV	NPV
ZN / culture	100	99	83	100
ZN / PCR dot-blot	23	99	23	100
	PPV	NPV	PPV	NPV
10%	PPV	NPV	PPV	NPV
ZN / culture	100	99	91	99
ZN / PCR dot-blot	38	98	39	99
	PPV	NPV	PPV	NPV
20%	PPV	NPV	PPV	NPV
ZN / culture	100	97	96	99
ZN / PCR dot-blot	58	98	59	98
	PPV	NPV	PPV	NPV
30%	PPV	NPV	PPV	NPV
ZN / culture	100	95	98	98
ZN / PCR dot-blot	71	93	71	96
	PPV	NPV	PPV	NPV
40%	PPV	NPV	PPV	NPV
ZN / culture	100	93	98	97
ZN / PCR dot-blot	79	89	79	94
	PPV	NPV	PPV	NPV
46%*	PPV	NPV	PPV	NPV
ZN / culture	99	88	98	96
ZN / PCR dot-blot	87	82	83	93

PPV, positive predictive value; NPV, negative predictive value. * the present study.

Considering anti-TB treatment status, there was a tendency toward a higher SE in the non-treated group as compared with previous TB cases in all tested methods, and SP was similar to that previously reported.⁷ ZN used in parallel with PCR showed SPs ranging from 83% to 86%, as previously described (84% to 87%) in developing countries using solely automated nucleic acid amplification (NAA) tests, and lower than those described (<95%) in industrialized countries.^{2,7,14} When different prevalence rates were simulated, high NPV was observed with a TB prevalence of 5-20%, characteristic of outpatient units and general hospital settings. However, these figures decreased in scenarios with a TB prevalence of >30%, especially among HIV⁺ subjects. As mentioned by other authors, in this report the sensitivity of the ZN staining was significantly lower among HIV⁺-TB patients, and the SE of both in-house PCRs was not influenced by the HIV status of the patient.^{2,15,16} These data confirm that the strategy of using ZN in parallel with PCR can be used for excluding TB in outpatient units and hospital settings, particularly in HIV⁻ subjects.

The lower SE of ZN when used in parallel with PCR (85%) may be a result of several factors. One of these is the presence of inhibitors that remained in the specimen after the extraction procedure; however, in our study the proportion of inhibitors (1.9%) was similar to those used in NAA tests (0.85% to 22.7%).^{14,17} Other factors may include a small number of unequally distributed mycobacteria in the test suspension owing to its division into three aliquots for the laboratory tests used in our study, or levels below the detection limit for in-house PCR (50 CFU).¹⁰ In fact, among the false negative results, 33.3% (11/33) of specimens were below the amplification test detection limit used for PCR. Additionally, the low copy number of IS6110 (insertion element) in the MTB is reported to decrease SE, but this has not been reported previously in Brazil.¹⁰ PCR demonstrated 22 false-positive results (including nine that had had TB in the past, one that presented a scar image in the chest X-ray that resembled inactive TB, five that were HIV⁺, and six that referred proximity with smear-positive PTB cases during the last six months). The value of the Kappa score obtained between the duplicates of PCRs was 100%. The strategy of associating ZN in parallel with culture showed the best performance in subjects infected or not by HIV; however, culture can delay diagnosis by three to six weeks, making the quick diagnosis of TB difficult. Therefore, the use of ZN in parallel with PCR may provide an alternative for the rapid diagnosis of TB, particularly among HIV⁺ individuals or those with atypical presentation and/or co-morbidities, where diagnosis delay may be lethal, and is critical for the prompt ini-

tiation of anti-TB treatment.¹⁷ Additionally, this strategy could reduce the risk of dissemination to other hospitalized patients and healthcare personnel.

In our study, the combination of ZN and PCR showed a great improvement in SE and LR-. Thus, the use of ZN and PCR may offer a novel approach for ruling out PTB cases, especially among HIV- subjects, not previously treated for TB, attended in hospitals in developing nations. In-house PCR is usually less expensive than automated nucleic acid amplification tests, and should be introduced more widely in developing nations after an evaluation of its cost-effectiveness and refined estimates of the likelihood of TB disease in different settings.

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