Direct susceptibility testing of *Mycobacterium tuberculosis* for pyrazinamide using the BACTEC MGIT 960 system

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Abstract

Pyrazinamide is a key antituberculosis drug, yet no rapid susceptibility test is commercially available. PZA drug susceptibility testing (DST) was performed directly on the sputum from 327 patients and compared with the indirect method using the BACTEC MGIT 960 system in the context of patient screening for participation in a drug trial. Compared to standard indirect PZA DST, direct DST was only successful in 59% of cases, but results obtained were highly accurate and available faster. Agreement between the direct and indirect method varied from 90 to 100% in each laboratory. The median times for obtaining PZA results from the time the specimen was collected ranged from 11 to 16 days for the direct and 18 to 95 days for the indirect across laboratories. The direct method is accurate and reproducible across laboratories. It can be expected to accelerate results in more than 50% of cases but it cannot replace indirect DST for PZA. Phenotypic methods remain the gold standard for DST in drug trials. If future studies can optimize the method to decrease the number of uninterpretable results, direct MGIT DST could be the new phenotypic DST standard for clinical trials providing more rapid detection of resistance to new drugs in experimental regimens.

Keywords: Pyrazinamide, tuberculosis, drug resistance testing, liquid culture

Introduction

Pyrazinamide (PZA) is a key anti-tuberculosis (TB) drug that has recently been shown to substantially enhance the activity of the novel agents bedaquiline (BDQ) and pretomanid (PA-824, Pa) in murine models of TB (1-3) and Phase II studies (4-6). Novel regimens based on the BDQ-PZA and Pa-PZA building blocks do not include isoniazid (INH) and rifampin (RIF) and are thus suitable for treatment of multi-drug resistant TB (MDR-TB, defined as TB resistant to at least INH and RIF).

PZA resistance in subjects with TB susceptible to INH and RIF is rare, i.e., 2%–10% of non-MDR-TB cases in South Africa (7, 8) and elsewhere (9-11). In patients with MDR-TB, however, recent studies have found between 60% and 70% PZA resistance in South African trial centers (12). Clinical trials with a novel 3-drug regimen such as BDQ-Pa-PZA in MDR-TB patients would require confirmed PZA susceptibility because undetected PZA resistance exposes participants to the risk of acquisition of resistance to the other agents in the tested regimen.

Although rapid molecular susceptibility tests detecting critical mutations directly on sputum samples are available for most first-line and the most important second-line agents, there is no commercial test for the rapid molecular detection of PZA resistance. The association of multiple mutations throughout the *pncA* gene with PZA resistance makes it difficult to design/develop a test for detecting PZA resistance (13). Phenotypic PZA testing in liquid culture medium is well established in clinical practice but lacks accuracy and reproducibility (14). Most reports cite problems of false PZA resistance detection with the MGIT 960, which is attributed to the inoculum concentration being too high (13). Another limitation of the phenotypic method is the long time to completion (15). This is due to the need to first grow up a primary culture and then grow a secondary culture with PZA at the required concentration

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to determine phenotypic susceptibility. As an alternative to the indirect method, the test can be set up directly from the clinical specimen. This eliminates the initial culture thus speeding up the availability of test results, but such abbreviated procedure can lead to invalid results due to culture contamination or insufficient growth if the inoculum contains too few viable bacteria (15, 16). This method has been evaluated for INH and RIF but not yet for PZA.

We investigated whether PZA testing via the automated BACTEC MGIT 960 liquid culture system (Becton Dickinson Diagnostic Systems, Sparks, MD) inoculated directly from sputum specimens is feasible, accurate and expedites the availability of PZA susceptibility results compared to the standard indirect method.

Materials and Methods

Patient specimens and ethical approval

Spot sputum specimens were collected from patients screened for eligibility to participate in a multicenter Phase II trial of a novel anti-TB regimen containing PZA (6). Patients were adults from community clinics with newly diagnosed smear-positive pulmonary TB and no apparent concomitant illness or conditions that would make participation inadvisable. Prior to the study, one laboratory tested 31 consecutive specimens to validate the direct MGIT DST for PZA. For the study, five mycobacteriology laboratories performed screening tests on sputum samples, among which were acid-fast bacilli (AFB) smear microscopy, Genotype MTBDR*plus* version 2 and MTBDR*sl* (Hain Lifescience, Nehren, Germany), and direct MGIT DST for PZA (Becton-Dickinson, Sparks, NJ). These screening tests were performed in parallel as capacity allowed as long as the patient was still considered for participation based on microbiological

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or clinical criteria. Consequently not all results were available for every subject. Although the direct MGIT for PZA DST was to be performed on one specimen, two of the labs tested additional specimens (Day -2, Day -1). Also, the intention was to test only smear-positive specimens; however, smear-negative specimens were tested, as the smear results were not always available before setting up the direct DST. The institutional review boards of all the participating sites approved the study. Written informed consent for study participation was obtained from all patients.

BACTEC MGIT drug susceptibility testing methods

Direct and indirect PZA susceptibility testing were performed as described by Siddiqi *et al* and manufacturer's instructions, respectively (15, 17). For the direct method, the sputum specimens were processed using the N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method at a final concentration of 1-1.5% NaOH. The remaining pellet was resuspended in phosphate buffer (pH 6.8), up to a final volume of 2 ml and was used as the inoculum for PZA susceptibility testing. The resuspended pellet was diluted 1/10 and 0.5 ml was inoculated into the control tube (also containing PANTA and the PZA enrichment supplement), while 0.5 ml of undiluted resuspended pellet was inoculated into the 100 µg/ml PZA containing tube (also containing PANTA and the PZA enrichment supplement). Tubes were incubated in the BACTEC 960 MGIT instrument, following the 21-day protocol for PZA susceptibility testing (17). Direct DST results from the MGIT instrument were recorded as susceptible, resistant or uninterpretable. Indirect DST results were recorded as susceptible or resistant, since tests with uninterpretable results were repeated until valid results were obtained. If the direct or indirect PZA result was resistant, the PZA tube was checked visually for evidence of contamination and a Ziehl-Neelsen stain and/or blood agar plate were performed to rule out contaminants. If contaminants were found, the result was reported as uninterpretable.

Uninterpretable results were therefore classified as contaminated (including X400 errors reported by the MGIT instrument), growth failure (X200 errors due to insufficient growth, i.e., the growth units of the control did not reach 400 within 21 days), or instrument failure.

Data analysis and statistics

The indirect result was regarded as the gold standard. Although there was a laboratory protocol, variations were observed amongst laboratories in the number and timing of direct and indirect tests performed. Laboratory 4 had duplicate indirect PZA results; only one result was considered for agreement analysis since duplicate indirects all gave the same results. For Laboratories 2 and 5, directs were repeated up to 3 times on different screening specimens: only the pair where both direct and indirect tests were done on the same specimen was kept. For Laboratory 4, directs were done on a separate specimen than indirects; directs were done once and indirects were repeated up to 2 times and paired as described above. No duplicates were done for directs nor indirects in Laboratory 3.

In order to calculate the direct MGIT success rate (reportable results), the reproducibility of replicate direct MGIT results, and the time to direct and indirect DST, all test results were used. To calculate the agreement between the direct and indirect tests, the results were paired as described above. The time between the specimen collection date and the ultimate PZA result date was calculated regardless of whether the result was interpretable or not. No times were available for the validation study. All direct DSTs were performed within 48-72 hours of specimen receipt in the lab except for one laboratory. Sputum specimens were processed for MGIT culture within the same timeframe. However, the time from determining an *M. tuberculosis* positive MGIT culture to setting up the indirect PZA DST varied.

Category agreement was calculated by dividing the number of categorical result matches (susceptible/resistant) by total tested (18). Chi-square was used to compare proportions. Correlation was measured using Spearman rank correlation coefficient. SPSS software version 20 (SPSS Corporation, Chicago, Illinois) was used for all analyses.

Results

Performance

Validation was performed with 31 sputum specimens. Of these, 24 (77.4%) had reportable PZA results: 17 susceptible and 7 resistant, with an agreement of 100% between the direct and indirect methods. The seven uninterpretable results were due to growth failure in 6 (85.7%), and contamination in 1 (14.3%)(Table 1).

PZA susceptibility testing was performed on the sputum of 327 patients: 398 tests were performed by the direct method and 207 by the indirect (Table 1). The PZA direct results were uninterpretable in 163 (41.0%), varying from 23% to 66% among the five laboratories. Reasons for uninterpretable PZA direct results were growth failure in 67.5%, contamination in 31.9%, and instrument failure in 0.6% (for distribution among laboratories see Table 1). Of 398 direct PZA tests done, 348 had smear results available (87.4%): 36 were smear negative (10.3%) and were more likely to give an uninterpretable PZA direct result (33 uninterpretable; 91.7%) compared to 312 positive smear specimens (110 uninterpretable; 35.3%; chi-square= 42.4, $p<0.001$). This was mainly due to insufficient growth: 30 of the 33 uninterpretable results were due to 200X errors (91%). A correlation between smear grading

and proportion of uninterpretable PZA results was also observed (Spearman Correlation=0.298, p<0.001) (Figure 1).

Agreement and reproducibility

For all laboratories, an analysis of pairs (1 direct and 1 indirect per patient as described in Methods) revealed that PZA resistance was detected in 12/139 (8.6%) pairs by the direct method and in 13/139 (9.4%) pairs by the indirect method. Of these 139 pairs, 134 were in agreement and five were not, for a 96.4% category agreement percentage. Two of the discrepant results were direct resistant/indirect susceptible, while three were direct susceptible/indirect resistant (Table 1). No further testing was done to determine the true nature of discordance.

Two laboratories performed direct tests in duplicate or triplicate. One had 20 sets of duplicate results (15 S/S and 5 R/R), showing 100% concordance. The other laboratory had 69% concordant results (9/13: 1 S/S/S, 1 R/R/R, 7 uninterpretable (U)[5 U/U, 2 U/U/U]), 31% results with uninterpretable values (4/13: 1 S/S/U and 3 S/U/U) and no discordants. Only one laboratory performed indirect tests in duplicate: 11 results were concordant (1 R/R, 10 S/S), 1 had uninterpretable value (contaminated/susceptible) and no discordants.

Time to availability of results

The median times for each lab ranged from 11 to 16 days for the direct, compared to 18 to 95 days for the indirect (Table 1). Table 2 compares the number of direct PZA tests with results available (reportable or uninterpretable) 7, 14, 21 and 28 days after specimen collection. Variable times were observed with >96% of the results available at 21 days (i.e. the maximum

duration of the MGIT PZA protocol) for 3 of the 4 laboratories. Such comparison was not done for indirect PZA results since the indirect tests were often not set up in real time.

Discussion

In this multicenter clinical trial of a novel anti-TB treatment regimen we compared PZA resistance testing performed directly on sputum specimens from untreated patients with the indirect test using the BACTEC MGIT 960 system. This evaluation was done in the context of time pressures dictated by the need of patients to be evaluated for participation and started on treatment without delay. The observed category agreement between the direct and indirect method (the reference method or gold standard) was excellent, varying from 90 to 100% per laboratory. Only 5 discrepant results were observed of 139 pairs (3.6%), similar to the discordance rate observed for the direct testing of INH (4.9%) and RIF (3.9%) by Siddiqi et al (15). Reproducibility of the direct method was excellent, although the numbers are too small to compare and confirm differences.

Compared to standard indirect PZA DST, direct DST was successful in 59% (range across laboratories: 34%-77%) of cases. The reason(s) for the variable performance amongst all laboratories is inexplicable. Performance was exceptionally poor in one laboratory, with the number of uninterpretable results equally due to insufficient *M. tuberculosis* density and contamination. The drug susceptibility testing failures could be attributed to poor technique in processing the sputum specimens resulting in inadequately digested and decontaminated specimens. Re-suspending the sputum pellet is another critical step in ensuring even distribution of *M. tuberculosis* and representative sampling for smear microscopy and culture inoculation. This was the first time these laboratories performed the direct MGIT drug susceptibility test method and no on-site training was provided prior to performing the study.

The 59% feasibility rate is lower than reported in a recent study where direct susceptibility testing of *M. tuberculosis* for INH and RIF using the same MGIT system in four laboratories yielded reportable results in 85% of 360 AFB smear-positive sputum specimens (15). As reported by Siddiqi et al., the most frequent reason for our uninterpretable direct results was growth failure. In their study, a 4 to 21-day protocol was used instead of the standard 4 to 13-day protocol for the INH and RIF indirect tests, to allow more time for the growth control tube to reach the required 400 growth units for a valid test. The indirect PZA test protocol is 4 to 21 days; the extended incubation time allows more time for the *M. tuberculosis* to grow if the growth rate in the slightly acidified MGIT PZA medium is slower. The same protocol was used for the direct PZA test since it was not possible to adjust the instrument protocol, i.e., extend it beyond 21 days using the BACTEC MGIT EpiCenter which was not available in these labs. Slow growth of some *M. tuberculosis* strains in PZA medium may have been a cause for growth failures. More likely the reason for insufficient growth in the control was the inoculum density being too low. Although the inoculum for the control tube is a 1/10 dilution of the sputum pellet, instead of the more diluted 1/100 used in the indirect test, the concentration of viable *M. tuberculosis* may have been very low in some sputum specimens despite being smear-positive. Furthermore, it is possible that some strains had a delayed lag time before beginning replication and did not reach the threshold of detection before the end of the protocol.

Several approaches to decreasing the number of uninterpretable results can be considered. For the contaminated cultures, the amount of antimicrobial mixture (PANTA), which is added to the control and PZA-containing tubes, could be increased to enhance suppression of contaminants. To decrease growth failures, a lower dilution of the sputum sediment could be

evaluated as inoculum for the control, i.e., using 1/5 instead of 1/10 dilution. Since the number of *M. tuberculosis* in the sputum sediment is lower than that in a positive MGIT culture used for indirect testing, the proportion of organisms between the control and drug tubes should still be appropriate with the 1/5 dilution.

The median times for each laboratory for obtaining PZA results from the time of specimen collection ranged from 11 to 16 days for the direct, compared to 18 to 95 days for the indirect (Table 1). In three laboratories where the direct PZA test was set up within 3 days of specimen collection and results were often available before the end of the 21-day protocol the turnaround time was 21 days for ≥ 96% specimens (Table 2). The longer turnaround time in Laboratory 5 was due to the lab being busy and prolonging the set up of the direct test. Longer delays were observed for the indirect results when contaminated MGIT cultures had to be decontaminated, re-cultured, and pure *M. tuberculosis* growth obtained before repeat DST. Logistical problems, such as heavy workload volumes along with insufficient laboratory staff and accessibility to biosafety cabinets also contributed to the delay in setting up indirect DSTs. The time to obtain results after the test was set-up ranged from 10 to 16 days for directs, compared to 7 to 8 days for indirects. A longer result time for directs is expected with the inoculum density being lower, especially in the tests that do not reach the growth unit threshold by the end of the 21-day protocol. In the INH/RIF direct MGIT study (15), similar results were obtained: 8-14 days for directs and 6-10 days for indirects. However, in the Siddiqi study the uninterpretable results were not included in the time to positive analysis (final results). It is likely that uninterpretable results would have longer time to positive results. Our direct test results times, with and without uninterpretable results, are comparable to those reported for INH and RIF; suggesting that *M. tuberculosis* grows at the same rate in MGIT PZA medium as in the MGIT SIRE medium used for INH and RIF testing.

Phenotypic methods remain the gold standard for DST in clinical trials, and past and current trials depend on phenotypic testing of anti-TB drugs to ensure study participants are susceptible to the drugs they are receiving. Having reliable susceptibility results for the study drugs within the screening period, e.g., 2-3 days, would be a significant advance for clinical trials. Currently the mechanism or molecular basis of drug resistance is not known for some of the second-line drugs and new TB drugs like Bedaquiline, Sutezolid, Pretomanid [PA-824], and Delamanid. Furthermore, not all gene targets associated with resistance are known (e.g., INH, fluoroquinolones, and injectables). So until current molecular tests are improved or new ones developed, a rapid phenotypic method like the direct MGIT would be preferable to indirect MGIT. Phenotypic methods may be replaced in the future with molecular tests; however, until we know the relationship between the resistance mutations, minimum inhibitory concentrations (MICs), and clinical outcomes there will be a need for phenotypic testing to determine MICs . Rapid MICs determinations are possible with the direct MGIT method (unpublished data).

Our study being conducted in the context of a clinical trial was limited by the variation in number and timing of tests in the participating laboratories., However, our results show that once reportable results are obtained, they are reliable and can be obtained in different laboratories. Additional studies with PZA are needed to investigate whether the frequency of uninterpretable results can be decreased by optimizing the method and gain more experience with MDR-/XDR- (extensively drug resistant) TB sputum specimens. If future studies provide reproducible and conclusive data, direct MGIT DST could be the new phenotypic DST standard for clinical trials and clinical management, not only for PZA but also for the new drugs in clinical development.

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Conflict of interests

No conflict of interests has to be stated by any author.

References

1. **Williams K, Minkowski A, Amoabeng O, Peloquin CA, Taylor D, Andries K, Wallis RS, Mdluli KE, Nuermberger EL.** 2012. Sterilizing activities of novel combinations lacking first- and second-line drugs in a murine model of tuberculosis. Antimicrob Agents Chemother **56:**3114-3120.

2. **Tasneen R, Li SY, Peloquin CA, Taylor D, Williams KN, Andries K, Mdluli KE, Nuermberger EL.** 2011. Sterilizing activity of novel TMC207- and PA-824-containing regimens in a murine model of tuberculosis. Antimicrob Agents Chemother **55:**5485-5492.

3. **Nuermberger E, Tyagi S, Tasneen R, Williams KN, Almeida D, Rosenthal I, Grosset JH.** 2008. Powerful bactericidal and sterilizing activity of a regimen containing PA-824, moxifloxacin, and pyrazinamide in a murine model of tuberculosis. Antimicrob Agents Chemother **52:**1522-1524.

4. **Diacon AH, Dawson R, von Groote-Bidlingmaier F, Symons G, Venter A, Donald PR, van Niekerk C, Everitt D, Winter H, Becker P, Mendel CM, Spigelman MK.** 2012. 14-day bactericidal activity of PA-824, bedaquiline, pyrazinamide, and moxifloxacin combinations: a randomised trial. Lancet **380:**986-993.

5. **Diacon A, Dawson R, Van Niekerk C, Hutchings J, Murray S, Schall R, Burger D, Everitt D, Mendel C.** 2014. 14 Day EBA Study of PA-824, Bedaquiline, Pyrazinamide and Clofazimine in Smear Positive TB Patients. Abstract 97LB, abstr 21st Conference on Retroviruses and Opportunistic Infections, Boston, MA, March 2014,

6. **Dawson R, Diacon AH, Everitt D, van Niekerk C, Donald PR, Burger DA, Schall R, Spigelman M, Conradie A, Eisenach K, Venter A, Ive P, Page-Shipp L, Variava E, Reither K, Ntinginya NE, Pym A, von Groote-Bidlingmaier F, Mendel CM.** 2015. Efficiency and safety of the combination of moxifloxacin, pretomanid (PA-824), and pyrazinamide during the first 8 weeks of antituberculosis treatment: a phase 2b, open-label, partly randomised trial in patients with drug-susceptible or drug-resistant pulmonary tuberculosis. Lancet **385:**1738-1747.

7. **Louw GE, Warren RM, Donald PR, Murray MB, Bosman M, Van Helden PD, Young DB, Victor TC.** 2006. Frequency and implications of pyrazinamide resistance in managing previously treated tuberculosis patients. Int J Tuberc Lung Dis **10:**802-807.

8. **Mphahlele M, Syre H, Valvatne H, Stavrum R, Mannsaker T, Muthivhi T, Weyer K, Fourie PB, Grewal HM.** 2008. Pyrazinamide resistance among South African multidrug-resistant Mycobacterium tuberculosis isolates. J Clin Microbiol **46:**3459-3464.

9. **Ando H, Mitarai S, Kondo Y, Suetake T, Sekiguchi JI, Kato S, Mori T, Kirikae T.** 2010. Pyrazinamide resistance in multidrug-resistant Mycobacterium tuberculosis isolates in Japan. Clin Microbiol Infect **16:**1164-1168.

10. **Muthaiah M, Jagadeesan S, Ayalusamy N, Sreenivasan M, Prabhu SS, Muthuraj U, Senthilkumar K, Veerappan S.** 2010. Molecular Epidemiological Study of Pyrazinamide-

342 Resistance in Clinical Isolates of Mycobacterium tuberculosis from South India. Int J Mol Sci
343 **11:**2670-2680.
344 11. **Kurbatova EV, Cavanaugh JS, Dalton T, E SC, Cegielski JP.** 2013. Epidemiology of
345 pyrazinamide-resistant tuberculosis in the United States, 1999-2009. Clin Infect Dis **57:**1081-
346 1093.
347 12. **Diacon AH, Pym A, Grobusch MP, de los Rios JM, Gotuzzo E, Vasilyeva I, Leimane**
348 **V, Andries K, Bakare N, De Marez T, Haxaire-Theeuwes M, Lounis N, Meyvisch P, De**
349 **Paepe E, van Heeswijk RP, Dannemann B, Group TCS.** 2014. Multidrug-resistant
350 tuberculosis and culture conversion with bedaquiline. N Engl J Med **371:**723-732.
351 13. **Piersimoni C, Mustazzolu A, Giannoni F, Bornigia S, Gherardi G, Fattorini L.** 2013.
352 Prevention of false resistance results obtained in testing the susceptibility of Mycobacterium
353 tuberculosis to pyrazinamide with the Bactec MGIT 960 system using a reduced inoculum. J
354 Clin Microbiol **51:**291-294.
355 14. **Chedore P, Bertucci L, Wolfe J, Sharma M, Jamieson F.** 2010. Potential for erroneous
356 results indicating resistance when using the Bactec MGIT 960 system for testing susceptibility
357 of Mycobacterium tuberculosis to pyrazinamide. J Clin Microbiol **48:**300-301.
358 15. **Siddiqi S, Ahmed A, Asif S, Behera D, Javaid M, Jani J, Jyoti A, Mahatre R, Mahto D,**
359 **Richter E, Rodrigues C, Visalakshi P, Rusch-Gerdes S.** 2012. Direct drug susceptibility
360 testing of Mycobacterium tuberculosis for rapid detection of multidrug resistance using the
361 Bactec MGIT 960 system: a multicenter study. J Clin Microbiol **50:**435-440.
362 16. **Libonati JP, Stager CE, Davis JR, Siddiqi SH.** 1988. Direct antimicrobial drug
363 susceptibility testing of Mycobacterium tuberculosis by the radiometric method. Diagn
364 Microbiol Infect Dis **10:**41-48.
365 17. **Becton Dickinson and Company.** 2009. BD BACTEC™MGIT™ 960 PZA Kit For the
366 Antimycobacterial Susceptibility Testing of Mycobacterium tuberculosis. Package Insert
367 L005486JAA.

18. **Clark RB, Lewinski MA, Loeffelholz MJ, Tibbetts RJ.** 2009. Cumitech 31A. Verification and Validation of Procedures in the Clinical Microbiology Laboratory. ASM Press, Washington, DC.,

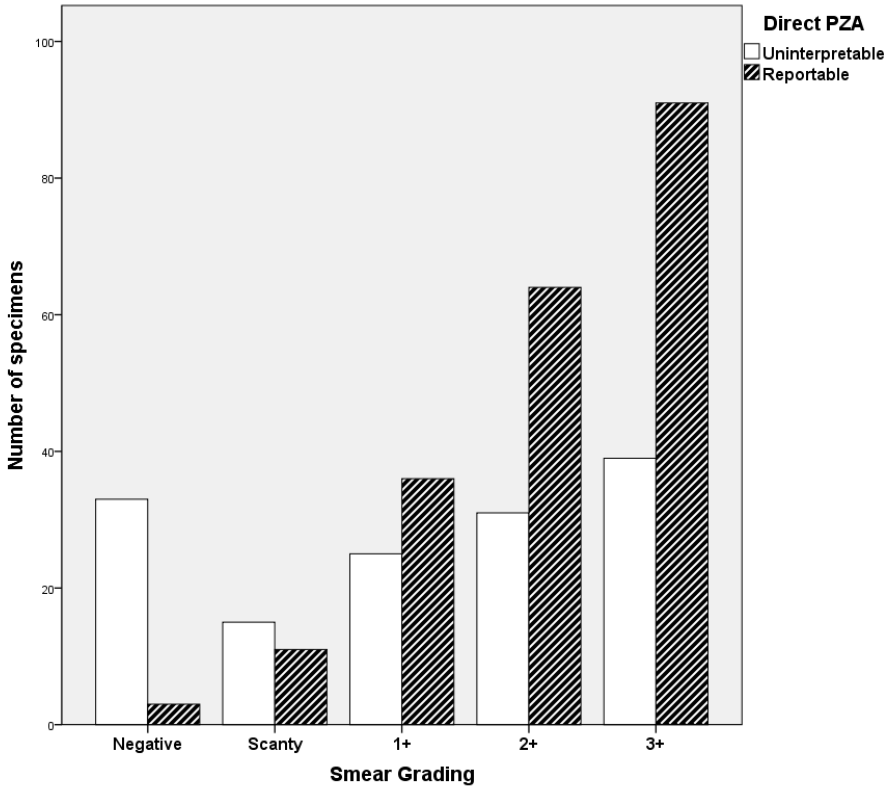
373 Table 1. Summary of direct and indirect PZA results

Findings	Laboratory 1 (validation study)	Laboratory 2	Laboratory 3	Laboratory 4	Laboratory 5	TOTAL (not including validation study)
No of patients	31	23	13	52	239	327
No of indirect PZA tests	31	23	13	37	140	207
No of direct PZA tests	31	47	13	51	287	398
Reportable direct PZA (% of directs done)	24/31 (77.4%)	16/47 (34%)	10/13 (76.9%)	30/51 (58.8%)	179/287 (62.4%)	235/398 (59.0%)
Uninterpretable direct PZA (% of directs done)	7/31 (22.6%)	31/47 (66%)	3/13 (23.1%)	21/51 (41.2%)	108/287 (37.6%)	163/398 (41.0%)
Causes of uninterpretable direct PZA (% of uninterpretable)						
X200 Error (growth failure)	6/7 (85.7%)	16/31 (51.6%)	2/3 (66.7%)	16/21 (76.2%)	76/108 (70.4%)	110/163 (67.5%)
Contamination	1/7 (14.3%)	15/31 (48.4%)	1/3 (33.3%)	5/21 (23.8%)	31/108 (28.7%)	52/163 (31.9%)
Instrument failure					1/108 (0.9%)	1/163 (0.6%)
DIRECT RESULTS						
Median Time: Collection date to Start PZA date in days (range)	N/A	0 (0-0)	1 (0-3)	0 (0-3)	2 (0-35)	

Findings	Laboratory 1 (validation study)	Laboratory 2	Laboratory 3	Laboratory 4	Laboratory 5	TOTAL (not including validation study)
Median Time: Start PZA date to PZA Result date in days for directs (range)	N/A	16 (3-29)	10 (7-21)	13 (5-25)	14 (1-25)	
Median Time: Collection date to PZA Result date in days (range)	N/A	16 (3-29)	11 (7-21)	13 (5-25)	16 (2-49)	
INDIRECT RESULTS						
Median Time: Collection date to Start PZA date in days (range)	N/A	88 (7-208)	29 (5-127)	48 (14-112)	11 (5-187)	
Median Time: Start PZA date to PZA Result date in days for indirects (range)[b]	N/A	7 (5-13)	7 (7-14)	8 (6-16)	7 (5-19)	
Median Time: Collection date to PZA Result date in days (range)	N/A	95 (14-213)	40 (12-141)	59 (21-126)	18 (11-195)	
No of pairs of direct/indirect (only interpretable results)	24	10	10	10	109	139
For these pairs, No of pairs in agreement	24 in agreement (17 S[c], 7 R[d]) 0 not in agreement	10 in agreement (8 S, 2 R) 0 not in agreement	10 in agreement (10 S, 0 R) 0 not in agreement	9 agreement (9S, 0 R) 1 not in agreement (direct R indirect S)	105 in agreement (97 S, 8 R) 4 not in agreement (1 direct R indirect S) (3 direct S indirect R)	134 in agreement (124 S, 10 R) 5 not in agreement (2 direct R indirect S) (3 direct S indirect R)
% agreement	100%	100%	100%	90.0%	96.3%	96.4%

[a] Proportion of reportable direct PZA that are either Smear Neg, Scanty, 1+ OR Smear 2+ 3+
[b] The difference between PZA Start Date and PZA Result Date does not include the time initially required to obtain a positive culture
[c] S = susceptible [d] R = resistant

Figure 1. Reportable and uninterpretable PZA direct results according to smear grading. Grading scale was based on WHO guidelines: Negative (0 colonies/100 fields), Scanty (1-9 colonies/100 fields), 1+ (10-99/100 fields), 2+ (1 to 10 AFB/field), or 3+ (more than 10 AFB/field).



386 Table 2. Number of direct PZA tests with results available (reportable or uninterpretable) 7,

387 14, 21 and 28 days after specimen collection.

		Direct PZA tests		
	Days	**# tests with results available**	**Cumulative**	**%**
Laboratory 2	7	12	12/47	25.5
	14	11	23/47	48.9
	21	22	45/47	95.7
	28	1	46/47	97.9
Laboratory 3	7	2	2/13	15.4
	14	9	11/13	84.6
	21	2	13/13	100.0
	28	0	13/13	100.0
Laboratory 4	7	8	8/48	16.7
	14	20	28/48	58.3
	21	18	46/48	95.8
	28	2	48/48	100.0
Laboratory 5	7	7	7/269	2.6
	14	89	96/269	35.7
	21	100	196/269	72.9
	28	66	262/269	97.4

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