ORIGINAL ARTICLE

Performance of a Novel Algorithm Using Automated Digital Microscopy for Diagnosing Tuberculosis

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Abstract

Rationale: TBDx automated microscopy is a novel technology that processes digital microscopic images to identify acid-fast bacilli (AFB). Use of TBDx as part of a diagnostic algorithm could improve the diagnosis of tuberculosis (TB), but its performance characteristics have not yet been formally tested.

Objectives: To evaluate the performance of the TBDx automated microscopy system in algorithms for diagnosis of TB.

Methods: Prospective samples from patients with presumed TB were processed in parallel with conventional smear microscopy, TBDx microscopy, and liquid culture. All TBDx-positive specimens were also tested with the Xpert MTB/RIF (GXP) assay. We evaluated the sensitivity and specificity of two algorithms—(*1*) TBDx-GXP (TBDx with positive specimens tested by Xpert MTB/RIF) and (*2*) TBDx alone—against the gold standard liquid media culture.

Measurements and Main Results: Of 1,210 samples, 1,009 were eligible for evaluation, of which 109 were culture positive for *Mycobacterium tuberculosis*. The TBDx system identified 70 specimens (68 culture positive) as having 10 or more putative AFB (high positive) and 207 (19 culture positive) as having 1–9 putative AFB (low positive). An algorithm in which "low-positive" results on TBDx were confirmed by GXP had 78% sensitivity (85 of 109) and 99.8% specificity (889 of 900), requiring 21% (207 of 1,009) specimens to be processed by GXP. As a stand-alone test, a "high-positive" result on TBDx had 62% sensitivity and 99.7% specificity.

Conclusions: TBDx used in diagnostic algorithms with GXP provided reasonable sensitivity and high specificity for active TB while dramatically reducing the number GXP tests performed. As a stand-alone microscopy system, its performance was equivalent to that of a highly experienced TB microscopist.

Keywords: microscopy; TBDx; triage; tuberculosis; Xpert

At a Glance Commentary

Scientific Knowledge on the Subject: The microbiological diagnosis of tuberculosis (TB) has improved dramatically with the introduction of the Xpert MTB/RIF (GXP) assay, an automated molecular test. However, the use of GXP is limited in high-burden countries because of its cost, meaning that TB diagnosis still relies on conventional smear microscopy, which may miss half of all cases.

What This Study Adds to the Field: The TBDx automated microscopy system is a novel TB test that relies on digital evaluation of high-throughput microscopic images and does not require a skilled microscopist. Use of the TBDx system to screen specimens prior to GXP could detect 90% of patients with GXP-positive TB while reducing the number of GXP tests required by 73%. Use of the TBDx system as a stand-alone tool can deliver performance equivalent to two highly skilled microscopists without the need to hire such personnel.

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An estimated 1 billion people have died of tuberculosis (TB) in the past 200 years, making it the leading infectious killer in known history (1). Despite this shameful history, the diagnosis of TB has relied, until recently, on sputum smear microscopy. This is a technique that is nearly 100 years old and misses half of all people with active TB, with operator-dependent sensitivities ranging between 30 and 80% in different epidemiological settings (2, 3).

In the past decade, important advances have been made in TB diagnosis, notably the introduction of the Xpert MTB/RIF assay (GXP; Cepheid, Sunnyvale, CA), which has a sensitivity for pulmonary TB of 89% (approaching 100% among those with smear-positive TB, but reduced to 68% in individuals with negative smears) and a specificity of 99% (4).

The cumulative worldwide volume of GXP tests conducted has reached over 6.2 million since its wide implementation in 2011 (5). However, this is far short of the estimated 77.6 million microscopy tests performed annually in the public sector of just the 22 countries with high TB burden (6). One of the primary reasons for incomplete uptake of GXP is its cost ($9.98 per cartridge under concessional pricing) (7), which in most settings is several-fold higher than that of microscopy (6) on a per-test basis and well beyond the existing TB budgets of most high-burden countries (8). Diagnostic "triage" algorithms that can reduce the number of GXP tests conducted without substantially lowering diagnostic performance are now an important priority (9).

Automated microscopy systems for TB diagnosis have the potential to fill this important diagnostic niche. Use of microscopy as a platform has the advantage of very durable, low-cost equipment that is already available for TB diagnosis throughout the world (6). Use of automated systems (rather than human eyes) to read microscopic slides can reduce subjectivity in results and potentially improve performance. The TBDx automated microscopy system (Signature Mapping Medical Sciences, Leesburg, VA) is a computer-aided detection system that automatically recognizes and counts images of putative acid-fast bacilli (AFB) in digitized fields of view (FOV).

In a previous proof-of-concept publication (10), researchers evaluating an earlier version of the TBDx system showed sensitivity of 49% and specificity of 98.9% when it was combined in a test-and-confirm algorithm with human microscopic confirming slides with one to nine AFB on the TBDx system. Use of TBDx in this triage approach reduced the workload of microscopists by 47%. However, the researchers in that study noted that the use of GXP, rather than manually read microscopy, as a confirmatory test may improve the sensitivity of the TBDx-based algorithm without compromising specificity.

On the basis of these early findings, improvements to the system have been made by the inclusion of a stepwise classification algorithm which performs post-processing categorization of false-positive (FP) objects. These FP objects are automatically removed based on a binary Boolean decision tree, with the consequence of improving specificity of the algorithm. Further details of this process have been presented elsewhere (11).

The aim of this study was to examine such a screening confirmation algorithm using a combination of TBDx and GXP. The primary objective was to evaluate the performance of algorithms using the latest version of the TBDx system as a screening test, confirmed by GXP, in an operational laboratory setting. A secondary objective was to evaluate the latest version of the TBDx system as a stand-alone tool to replace conventional smear microscopy in resource-constrained settings. Some of the results of these studies have been reported previously in the form of an abstract (12).

Methods

Setting

The study was conducted at the National Tuberculosis Reference Laboratory within the Centre for Tuberculosis at the National Institute for Communicable Diseases, Johannesburg, South Africa. Approval for the study was received from the Faculty of Health Sciences Research Ethics Committee at the University of Pretoria.

Samples and Study Procedure

This study was nested within an ongoing TB surveillance program. Consecutive sputum samples from adult patients (age $\geq$ 18 yr) with presumed TB and not receiving treatment for TB were obtained over a 2-week period. These sputum samples (one per patient) were digested and decontaminated as previously described (13), and sediments were then processed for auramine-stained smear microscopy, read both manually and with the TBDx automated system, as well as for liquid medium culture for mycobacteria (BACTEC MGIT 960 mycobacteria growth indicator tube; BD, Sparks, MD). The remnant sediments of processed samples were then stored at 2–8°C. If the TBDx system identified one or more putative AFB in images of 300 FOV, the remnant sediment was resuspended in the GXP sample reagent at a 2:1 ratio and analyzed using the GXP (14).

Laboratory Testing

Auramine-stained smear microscopy (reading 100 FOV) and TB culture were performed as previously described (15), and smear grading was done following the International Union Against Tuberculosis and Lung Disease (IUATLD) guidelines (16). All cultures recorded as positive by the MGIT 960 system were confirmed as *Mycobacterium tuberculosis* by demonstration of AFB on Ziehl-Neelsen staining and a positive MPT64 antigen result.

To ensure that the quality of microscopy was of a high standard, smears were read by a TB microscopist (G.N.) with more than 40 years of experience in AFB smear microscopy. All positive smears and at least 10% of negative smears from each batch of tests read by the microscopist were confirmed by a second highly experienced microscopist with similar experience. Any discordance was reviewed by the two microscopists again (blinded from the TBDx and GXP results), and a final result was decided by consensus. Sediments of specimens from which smears were assessed to be negative upon review as described above were retrieved and tested with the GXP assay as an additional quality assurance measure. These negative smears were in addition to the TBDx-positive sediments that were tested by GXP which formed part of the algorithm where only TBDx positives would be confirmed by GXP.

Once the microscopists had examined the smears, an independent technician transferred the slides containing the smears to a TBDx instrument cartridge for automated microscopy batch processing. Captured images of each of the smears on

the loaded slides were processed with the TBDx software to assess 100 FOV for each of the following areas on the smears: (*1*) along the perimeter of a rectangle with a length of 40 and width of 10 FOV (100 FOV) in the center of the slide, (*2*) four rows of 25 FOV (100 FOV) elsewhere on the slide, and (*3*) two rows of 50 FOV (100 FOV) elsewhere on the slide. The results were then reported as either putative AFB positive or AFB negative for each set of 100 FOV and the aggregated set of 300 FOV. Each digital FOV represents approximately 33% of an optical FOV seen by a microscopist. Acquiring 300 digital FOV in this study ensures an equivalent sampling area by the camera and the microscopist (10). For putatively positive smears, grading was also performed using the IUATLD criteria (using 300 automatically read FOV as equivalent to 100 manually read FOV). The operator of the TBDx was independent and blinded from the routine results, even though the TBDx results were computer generated.

Statistical Methods

"TBDx positive" was defined as at least 1 AFB seen in 300 FOV. For the analysis, we defined, using *a priori* criteria, "high positive" by TBDx as those slides with 10 or more putative AFB per 300 FOV and "low positive" as those with 1–9 putative AFB per 300 FOV.

We conducted three separate analyses, each to evaluate a different potential algorithm. The first two analyses addressed the primary objective. In the first analysis, we assumed that all specimens judged as high positive or low positive by TBDx would be confirmed by GXP. In the second analysis, we assumed GXP confirmation only of low positive specimens. In this analysis, there would be no drug susceptibility results for TBDx high positive specimens.

In the third analysis, to address the secondary objective, TBDx was used as a stand-alone test, with low positive results treated as negative. Drug susceptibility results, if available, were ignored. We further stratified these analyses with and without the inclusion of specimens having only one putative AFB detected by the TBDx system.

The culture results were used as the reference for all assessments of diagnostic performance. For sensitivity calculations, the denominator was the number of specimens for which the culture was positive for *M. tuberculosis*; for specificity calculations, the denominator was the number of specimens for which the culture was negative. Likelihood ratios were also calculated. Contaminated cultures or cultures positive for nontuberculous mycobacteria were excluded. The binomial exact method was used for calculation of confidence intervals.

Results

As part of the quality assessment, a GXP assay was done for all 277 results that were TBDx positive and on 127 that were TBDx negative. The GXP assay was repeated for 27 of the 28 with an error or an invalid result (1 had insufficient specimen volume for a repeat). The result was positive in 5% (6 of 127) of the TBDx negatives; 2 were low positive, and 3 were very low positive. Among the TBDx positives, the GXP was positive in 35% (96 of 277) of cases.

Performance Characteristics of Routine Smear

Of 1,210 sputum specimens available for the study, 1,009 (83.5%) were eligible for analysis (Figure 1). Of these, 109 were culture positive for *M. tuberculosis* (10.8%). The microscopists graded 75 of these 109 as smear positive, resulting in a sensitivity of 68.8% (95% confidence interval [CI], 59.2–77.3%) (Table 1). The remaining 900 were culture negative (89.2%), of which the microscopists graded 7 as smear positive, giving a specificity of 99.2% (95% CI, 98.4–99.7%).

Number of AFB Detected by the TBDx System Compared with Culture

The proportion of TBDx-positive specimens that were also *M. tuberculosis* culture–positive ranged from 7 (4.4%) of 158 smears where only 1 AFB was detected by TBDx to 68 (97.1%) of 70 smears with 10 or more AFB detected by TBDx (Table 2). Of the two specimens that had 10 or more AFB detected by TBDx that were culture negative, one was positive in GXP.

Performance Characteristics of TBDx-GXP Algorithm

The performance characteristics of the different combinations and number of GXP tests required for confirmation are shown in Table 3. An algorithm in which all TBDx high positive and low positive results were confirmed with GXP had a sensitivity of 77% (84 of 109; 95% CI, 68–85%) and specificity of 98.9% (890 of 900; 95% CI, 98.0–99.5%), requiring 277 GXP assays to be run on a total sample size of 1,009 people with presumed TB. If TBDx cases with only 1 putative AFB were regarded as negative, the number of GXP assays to be run was lowered to 119, with a slightly increased specificity (99.4% [895 of 900];

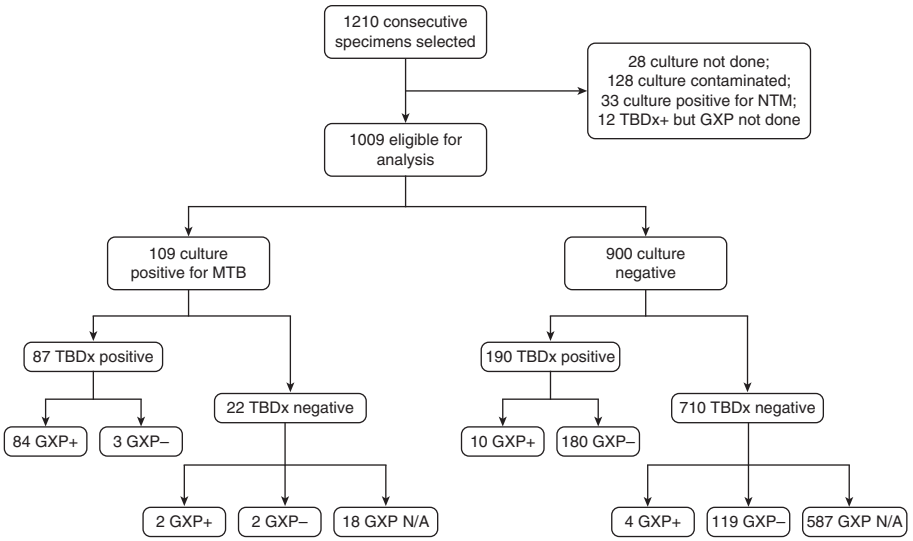

Figure 1. Flowchart showing eligibility for analysis and results of culture, TBDx, and the Xpert MTB/RIF assay. GXP+ = positive on Xpert MTB/RIF; GXP− = negative on Xpert MTB/RIF; GXP N/A = Xpert MTB/RIF not done; MTB = *Mycobacterium tuberculosis*; NTM = nontuberculous mycobacteria.

Table 1. Frequency and Percentage Distributions of Microscopist's Smear Status (Based on Reading 100 Fields of View) by Culture Results in the 1,009 Specimens

Microscopist's Reading*	Cultures Positive for *Mycobacterium tuberculosis* (*n* = 109)	Negative Cultures (*n* = 900)
Smear negative	34 (31.2%)	893 (99.2%)
Scanty positive	3 (2.8%)	1 (0.1%)
1+ positive	10 (9.2%)	3 (0.3%)
2+ positive	18 (16.5%)	2 (0.2%)
3+ positive	44 (40.4%)	1 (0.1%)

*Scanty: 1–9 acid-fast bacilli (AFB)/100 fields of view (FOV); 1+: 10–99 AFB/100 FOV; 2+: 1–9 AFB/FOV; 3+: >10 AFB/FOV.

95% CI, 98.7–99.8%), but sensitivity dropped by 5% (72% [78 of 109]; 95% CI, 62–80%). The five culture-negative specimens that would be classified as positive by this algorithm included four that were smear positive and five that were positive on GXP (three classified as "medium" and two as "high"), suggesting that true specificity could be even higher.

If TBDx high positive results were taken as positive (without GXP confirmation) and only low positive results confirmed by GXP, sensitivity was 78% (85 of 109; 95% CI, 69–85%) and specificity was 98.8% (889 of 900; 95% CI, 97.8–99.4%), with only 207 GXP assays required. Thus, relative to the "confirm all positives" algorithm, confirming only low positives with GXP led to one additional true-positive and one additional FP diagnosis, whereas confirming only those with 2–9 putative AFB per 300 FOV led to five fewer true-positive and four fewer FP diagnoses.

Performance Characteristics of TBDx as a Stand-Alone Tool

The TBDx, when used as a stand-alone tool, showed an overall sensitivity 80% (87 of 109; 95% CI, 71–87%) and a specificity of 78.9% (710 of 900; 95% CI, 76.1–81.5%) when we included one putative AFB as positive. The highest specificity was observed when only TBDx high positive slides were assumed positive (and those with zero to nine AFB assumed negative) at 99.8% (898 of 900; 95% CI, 99.2–100.0%), but sensitivity was 62% (68 of 109; 95% CI, 53–71%). The positive likelihood ratio for the latter algorithm was 310 and the negative likelihood ratio was 0.38 compared with 3.8 and 0.25, respectively, for scenarios where any putative AFB on TBDx was regarded as positive. In the algorithm where all cases with two or more AFB detected by TBDx were assumed positive (and those with zero or one AFB assumed negative), the sensitivity was 11% higher at 73% (79 of 109; 95% CI, 64–81%) and specificity at 95.7% (861 of 900; 95% CI, 94.1–96.9%). This is the same sensitivity as the algorithm with GXP confirmation of specimens with two to nine AFB detected by TBDx; however, in this algorithm, the specificity was 99.3% (894 of 900; 95% CI, 98.6–99.8%) but required only 49 GXP assays (5%) in 1,009 patients.

Table 2. The Distribution of the Number of Putative Acid-Fast Bacilli Detected by TBDx on 300 Fields of View and Relationship to Culture Positivity for *Mycobacterium tuberculosis*

Number of Putative AFB Detected by TBDx	Overall [*n (Column %)*]	Cultures Positive for *M. tuberculosis* [*n (Row %)*]
0	732 (72.5%)	22 (3.0%)
1	158 (15.7%)	7 (4.4%)
2	26 (2.6%)	5 (19.2%)
3	7 (0.7%)	2 (28.6%)
4	8 (0.8%)	3 (37.5%)
5–9	8 (0.8%)	2 (25.0%)
10–99	14 (1.4%)	14 (100.0%)
100–999	26 (2.6%)	25 (96.2%)
1,000+	30 (3.0%)	29 (96.7%)
Total	1,009 (100.0%)	109 (10.8%)

Definition of abbreviation: AFB = acid-fast bacilli.

Discussion

Automated microscopy has the potential to improve the diagnosis of TB, either by reducing the volume of expensive confirmatory tests required (where such tests are performed) or by reducing the need for trained microscopists (where more expensive tests are unavailable). This evaluation demonstrates that the improved TBDx system achieved both of these outcomes with good overall performance. Specifically, an algorithm in which GXP is used to confirm low positive results by TBDx can achieve a sensitivity for active pulmonary TB of 78% and specificity of 98.8%, with only one in five specimens requiring GXP testing. Without GXP confirmation, TBDx alone achieved sensitivity and specificity similar to those of two highly experienced microscopists working in tandem with the added benefit of processing a large number of slides without the need for trained personnel. These results, if confirmed in other epidemiological settings, suggest that TBDx can substantially improve diagnostic algorithms for TB by reducing resource requirements without substantively compromising diagnostic accuracy.

The World Health Organization (WHO) has released target product profiles for new diagnostics for TB, one of which is for a triage tool (17). The minimal specification is a sensitivity greater than 90% compared with the confirmatory test (with an ideal specification of 95%) and specificity greater than 70% (ideal >80%). Using GXP as the confirmatory test, TBDx had a sensitivity near 90% and a specificity of near 80% in this study. We have demonstrated the value of this approach, with targeted use of confirmatory tests in a higher-prevalence subgroup resulting in excellent performance and reduced risk of FPs with the second diagnostic.

Importantly, the vast majority of culture-negative samples (79%) were eliminated as negative by TBDx, allowing

Table 3. Performance Characteristics of TBDx-Alone and TBDx/GXP Algorithms, by Number of Putative Acid-Fast Bacilli Detected on 300 Fields of View, Compared with Gold Standard of Culture for 1,009 Smears

	Sensitivity (95% CI)	Specificity (95% CI)	LR+	LR−	GXP Tests Required
TBDx without GXP confirmation					
Low and high positive					
⩾1 putative AFB	80% (71–87%)	78.9% (76.1–81.5%)	3.8	0.25	N/A
>1 putative AFB	73% (64–81%)	95.7% (94.1–96.9%)	17.0	0.28	N/A
High positive only					
⩾10 putative AFB	62% (53–71%)	99.8% (99.2–100.0%)	310.0	0.38	N/A
TBDx with GXP confirmation of only low positive TBDx results					
Low and high positive					
⩾1 putative AFB	78% (69–85%)	98.8% (97.8–99.4%)	65.0	0.22	207
>1 putative AFB	73% (63–81%)	99.3% (98.6–99.8%)	104.3	0.27	49
TBDx with GXP confirmation of any high or low positive TBDx results					
Low and high positive					
⩾1 putative AFB	77% (68–85%)	98.9% (98.0–99.5%)	70.0	0.23	277
>1 putative AFB	72% (62–80%)	99.4% (98.7–99.8%)	120.0	0.28	119
High positive only					
⩾10 putative AFB	62% (52–71%)	99.9% (99.4–100.0%)	620.0	0.38	71

Definition of abbreviations: AFB = acid-fast bacilli; CI=confidence interval; GXP = Xpert MTB/RIF; high positive = AFB ⩾10 putative AFB; low positive = 1–9 putative AFB; LR+ = positive likelihood ratio; LR− = negative likelihood ratio; N/A = Xpert MTB/RIF not done.

effective use of a more accurate and expensive diagnostic tool as a reflex in cases with a higher likelihood of TB. A study done in Pakistan and another in South Africa (18, 19) have demonstrated that GXP performs very well relative to microscopy in these programmatic scenarios; however, the overall yields at a population level were low, with 13% of GXP tests in Pakistan and 8% in South Africa being positive. The use of a triage test such as TBDx in these algorithms might therefore have dramatically reduced the number of GXP tests performed, without substantial loss of sensitivity. Furthermore, a very high positive likelihood ratio was observed when TBDx was confirmed by Xpert, with a modest negative likelihood ratio.

The algorithm where only cases with two to nine putative AFB detected by TBDx were confirmed by GXP had sensitivity of 73% and specificity of 99.3% and required only 49 GXP tests in 1,009 patients. This provides a valuable middle-ground solution to improve diagnostic yields over conventional microscopy to a level comparable to that of other molecular tests (20) in resource-constrained settings. In this scenario, with a positive likelihood ratio of 104.3, if the pretest probability for TB disease is only 20% for an individual patient, the posttest probability of TB disease of the positive result will be above 95%, whereas a negative result would have a 5% posttest probability of TB disease for a negative result.

A limitation of this algorithm is the lack of rifampicin resistance results in all microscopy-positive cases with more than 10 AFB per 300 FOV. Conducting a GXP test in these cases for the sake of a rifampicin resistance result would come at a commensurately higher cost, which countries could justify or refuse based on existing budgets. Thus, in the African and Southeast Asian regions where the prevalence of multidrug resistance is below 2.5% in new cases of TB, this limitation may be less of a concern, and reflex testing in this subgroup could be reserved for patients with risk factors for drug-resistant TB or those for whom initial first-line therapy has failed.

A recent diagnostic landscape report (21) has shown a large array of new technologies nearing market entry. These technologies face two fundamental challenges—namely, affordability and performance variability across tested populations—that may limit the generalization of results. By contrast, as a triage test, TBDx can lower potential costs and reduce subjectivity compared with human assessments or interpretations. Novel studies applying such algorithms could fast-track these new technologies to fill an important global diagnostic gap.

TB disproportionately affects individuals in resource-limited settings, and the paradigm of new high-performance (and high-cost) diagnostics may not fit with the existing reality and sustainability concerns in resource-poor settings (22), suggesting that the practicalities of the real world present challenges for a wholesale move to molecular diagnostics. This has also been noted in a cost and affordability analysis whose authors stated that it would not be financially viable for low-income countries to adopt GXP as a primary test for all presumptive TB cases (8).

TBDx does offer a solution for addressing the human resources shortages in high-burden settings as a stand-alone system. It demonstrated excellent performance against a high microscopy standard consisting of two microscopists with a combined experience exceeding 80 years. The TBDx as a stand-alone diagnostic tool in the current version has shown improvement from the previous proof-of-concept version, with sensitivity increasing from 49% to 62% and specificity increasing from 98.9% to 99.7%, but importantly without the need for manual review. Unlike human readings, which have the risk of "change blindness" (23), computer-aided

systems such as the TBDx do not have these risks, which is the likely reason for the high consistency observed in each set of the 100 FOV of the 300 read by TBDx (data not shown). Further evaluation of the TBDx to improve the WHO quality assurance of routine microscopy through its rechecking program (8) should also be considered.

For TBDx with only one or two putative AFB observed, the likelihood of the culture's being positive is less than 5% (Table 2), highlighting the importance of confirming such results. As with manual microscopy, automated microscopy does not distinguish AFB on the basis of viability. Thus, it is possible that FPs detected by automated microscopy represent nonviable organisms in patients recently diagnosed who were on treatment and thus were not reported or that these organisms were in a nonreplicating persister state (24). However, only five of these FPs tested positive on GXP (which likewise detects DNA without assessment of viability), making it more likely that these specimens represent fluorescent artifacts. Importantly, apparent artifacts in automated microscopy can be visually adjudicated by human experts and may have clinical relevance.

The findings of this study, though positive, have several limitations. The study was conducted in a high HIV prevalence setting where microscopy is expected to perform poorly and is used at a single reference laboratory where the overall microscopy standard is high. We were also unable to stratify performance by HIV status, as these data were not available. The exclusion of pediatric cases limits the generalizability of our data to this population group; however, the performance of microbiological tests, including smear microscopy and GXP, is known to be poor relative to a clinical reference standard (25), and it is unlikely that the proposed algorithms would provide any further performance advantage for these cases.

In addition, all tests were performed on digested and decontaminated sputum samples, which is not generally the case in programmatic settings. In this study, we excluded from the analysis cultures that were contaminated (128 of 1,210); however, the cases identified by TBDx and confirmed by GXP in this subset are likely to be true cases and would potentially increase the true yield from the algorithms using a strict culture-only standard (excluding contaminated results). Last, in the present study, we used fluorescence microscopy rather than one of the new light-emitting diode–based microscopy systems, which may further improve microscopy detection rates (26). All of these limitations can be addressed in further studies.

Conclusions

The TBDx system demonstrated potential as a useful triage tool, with GXP used to confirm intermediate results. This novel system could also potentially serve as a triage test for other emerging technologies, ensuring that these tools are efficiently used while maintaining good performance. In our study, the technology as a stand-alone system proved comparable to that of a highly experienced microscopist and therefore offers a diagnostic solution that could provide quality-assured microscopy services in settings where trained microscopists are difficult to find. In either algorithm, the use of TBDx automated microscopy has the potential to improve upon the existing diagnostic standard of care. ■

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