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Revolutionizing TB Diagnosis: Intelligent Microscopy and Image Recognition for Enhanced Acid-Fast Bacilli Detection

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Abstract: Background: Microscopic examination of acid-fast stained sputum smears for detecting mycobacterial acid-fast bacilli (AFB) remains the most economical and readily available method for laboratory diagnosis of tuberculosis (TB). However, this conventional approach has limitations, including low sensitivity and labor-intensive procedures. **Methods**: An automated microscopy system incorporating artificial intelligence (AI) and machine learning for AFB identification was evaluated. The study was conducted at an Infectious Disease Hospital in Jiangsu Province, China, utilizing an intelligent microscope system (TB-Scan, Wellgen Medical, Kaohsiung). A total of 1,000 sputum smears were included in the analysis, with the system capturing digital microscopic images and employing an image recognition model to automatically identify and classify AFB. Referee technicians provided the gold standard for discrepant results. **Results**: The automated system demonstrated an overall accuracy of 95.00% (950/1,000), sensitivity of 91.24% (177/194), and specificity of 95.91% (773/806). Notably, the system identified 21 smears as positive that were previously reported as negative, with referee technicians confirming 17 of these as true positive and retracting the test results accordingly. Recalculating the performance, the accuracy increased to 96.70% (967/1,000), sensitivity to 91.94% (194/211), and specificity to 97.97% (773/789), with a false negative rate of 8.06% (17/211) and a false positive rate of 2.03% (16/789). **Conclusions**: The incorporation of AI and machine learning into an automated microscopy system demonstrated the potential to enhance the sensitivity and efficiency of AFB detection in sputum smears compared to conventional manual microscopy. This approach holds promise for widespread application in TB diagnostics and potentially other fields requiring labor-intensive microscopic examination.

Keywords: TB smears; AI; machine learning; TB diagnosis; STOP TB

Background

Tuberculosis is treatable, preventable, and curable. Sustained declines in tuberculosis deaths in many countries during the past 50 years provide evidence that ending the pandemic is foreseeable (Churchyard, 2017). However, tuberculosis, which has plagued humanity and has killed hundreds of millions of people over the past two centuries, remains a global public health threat. In 2023, 1.3 million people died from tuberculosis (95% UI: 1.18 – 1.43m), including 167,000 people with HIV, representing more deaths than any other infectious disease (WHO 2023). World leaders in the most recent United Nations High-Level Meeting (UNHLM) on TB made commitments and requests to address the global tuberculosis crisis (WHO, 2023), which included providing comprehensive care to all people with TB, addressing the crisis of drug-resistant TB, strengthening the engagement of civil society and communities affected by TB, and enabling and strengthening TB research. It highlights the need for comprehensive care, addressing drug-resistant TB, engaging civil society and communities, and promoting TB research. The commitments made during the meeting provide a strong impetus to accelerate the TB response and work towards ending TB.

The World Health Organization (WHO) recommends the acid-fast stain method of sputum smears as the most robust and economical method for the first line of laboratory diagnosis of pulmonary tuberculosis. This method relies on the microscopic examination of sputum samples for acid-fast mycobacteria bacilli (AFB). However, it is important to note that the sensitivity and specificity of smear microscopy are poor, as it only detects 10 to 75% of pulmonary TB cases. Additionally, smear microscopy is labor-intensive and tedious. While new molecular-based methods like Xpert MTB/RIF have become available, they have not been widely deployed in rural areas due to substantially higher costs and infrastructure constraints, which may not be affordable in many countries with high TB burden countries in the foreseeable future. Thus, despite advancements in molecular diagnostics, the acid-fast stain method remains the recommended first-line laboratory diagnosis for pulmonary tuberculosis due to its robustness and cost-effectiveness. Furthermore, WHO suggests the availability of 1.1 microscopy laboratories per every 100 thousand population to enhance the diagnostic capacity for tuberculosis (WHO 2023).

Recently, some automated TB smear microscopy systems have been developed that take advantage of artificial intelligence (AI) and big data analysis, which may significantly increase the sensitivity of TB smear microscopy (Lewis, 2012; Panicker, 2016; Xiong, 2018; Zingue, 2018; Lopez-Garnier, 2019). Such a system may include a motorized stage to load the smear slides into an automatic bright-field microscope. Then, the system performs auto-focus, digitally captures the smear images, analyzes the images, and classifies smear slides as positive or negative. Although all these studies reported better performance than human examination, most are still in development or just “proof-of-concept” systems. Until 2022, a commercialized system for automatic detection of AFB has received medical device registration in several countries (Huang, 2022; Fu, 2023). This is a continuation study to describe the performance characteristics and medical technician’s workload of a diagnostic algorithm for the identification of AFB under a microscope using image recognition technology.

Materials and Methods

Study Hospital: The Study Hospital was formerly an infectious diseases specialty hospital located in Southern Jiangsu, China. The hospital has 900 beds, of which 210 are in the respiratory department. An average of 80 smears are tested for mycobacteria in the laboratory. At least three technicians are on duty daily to perform TB smear microscopy.

Specimen: This study initially included 1,150 smears. One hundred fifty smears were rejected due to incomplete stain removal (n=60), smear location shift (n=8), smear too thick (n=3), smear too thin (14), smear dropped off (n=4), and slide size too big or too small for the system (n=21). The remaining 1,000 smears were enrolled.

Procedures: An automated smart medical microscope system (“system”) (TB-Scan, Wellgen Medical, Kaohsiung) was installed in a negative pressured isolation laboratory. The system consists of two components: (1) microscopic imaging acquisition hardware with auto-focusing and slide-scanning capability to cover the 1cm by 2cm specimen based on WHO recommendation (300 fields @1,000x oil lens); (2) image recognition algorithm for detection and classification of positive AFBs. After the microscopic images were digitally captured and stored, candidate AFBs were detected and marked from other substances and tissues in the smear based on color and morphological features. These candidate AFBs were processed by a proprietary software classifier. The results are recorded as positive if any AFB was identified in the image of the slide. Laboratory technician supervisor served as the Gold Standard in evaluating the system's performance.

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Quality Control: All positive smears detected by TB-Scan were re-examined by a microscope (Olympus CX-21) under a 1,000x oil lens to verify, and microscopic images were captured and stored by a cellphone (iPhone 13, Apple Inc. CA)

Data Interpretation: Test performance evaluation is based on sensitivity and specificity. Sensitivity (also called the true positive rate) measures the proportion of positives correctly identified as such (e.g., the percentage of positive TB smears correctly identified from the true positives). Specificity (also called the true negative rate) measures the proportion of actual negatives correctly identified as such (e.g., the percentage of negative TB smears correctly identified as not having the condition).

Results

Based on the original hospital clinical records, there were 194 AFB-positive smears and 806 AFB-negative smears. Based on TB-Scan's results, there were 210 AFB-positive smears and 790 AFB-negative smears. Of the 210 AFB-positive smears by TB-Scan, 198 smears contained AFB under microscope examination, and AFB was not found in the remaining 12 smears.

Based on the results mentioned above, the confusion matrix is as follows

Test Performance		Gold Standard	
		Positive	Negative
TB Scan	Positive	**177**	**33**
	Negative	**17**	**773**

The accuracy is 95.00% (950/1,000), sensitivity 91.24% (177/194), specificity 95.91% (773/806), false negative rate 8.76% (17/194) and false positive 4.09% (33/806). However, 21 smears were previously reported as negative, but both TB-Scan positive and the microscopic images were found positive. After showing the images to the medical technician in the study hospital (our Gold Standard), the technician ruled out four smears and maintained her judgment as negative, and agreed that the remaining 17 smears should have been recorded as positive. Therefore, the confusion matrix was re-calculated as follows:

Test Performance		Gold Standard	
		Positive	Negative
TB Scan	Positive	**194**	**16**
	Negative	**17**	**773**

The accuracy is 96.70% (967/1,000), sensitivity 91.94% (194/211), specificity 97.97% (773/789), false negative rate 8.06% (17/211) and false positive 2.03% (16/789).

Discussion

The most economical, rapid, and readily available method for laboratory diagnosis of TB is acid-fast staining of sputum smear to identify mycobacterial acid-fast bacilli (AFB). However, the sensitivity of smear microscopy is highly variable (Steingart, 2006) due to less experienced or trained staff, long hours workload, and no presence of quality assurance (Nguyen, 1999; Van Deun 2002). New technologies, such as the Xpert and TB-LAMP, based on molecular methods are becoming available. It is unlikely that these technologies will be affordable replacements for smear microscopy in many high-burden countries without subsidy from WHO or Gates Foundations. Thus, if automation, AI, and machine learning can be applied to TB smears, such a system may significantly increase the sensitivity of TB smear microscopy.

In this on-site test, Wellgen's TB-Scan achieved accuracy of 96.70% (967/1,000), sensitivity of 91.94% (194/211), specificity of 97.97% (773/789), false negative rate of 8.06% (17/211) and false

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positive of 2.03% (16/789), more than 90% in test sensitivity and specificity, well above its previous studies. Due to more consistent specimen preparation, the overall detection performance was better than the previous two studies (Huang, 2022; Fu, 2023). This is competitive with Xpert, which has a sensitivity of around 90% as well (WHO, 2023). In addition, regardless of the costs and resource issues with molecular methods, TB smear microscopy continues to play a role in TB diagnosis in monitoring the treatment of TB cases (WHO, 2023).

It is noteworthy that 17 smears were false negatives based on TB-Scan analysis. After carefully examining each scanned image, both technicians in this study could not find images with AFB. The smear that contains AFB may be outside of the scan area. Thus, to minimize such false negative results, smear preparation should follow a standardized procedure, and the specimen area should be in accordance with TB-Scan's scan area.

When considering the field deployment of an automated microscope system for clinical laboratories, several issues are noteworthy and could be considered as weaknesses: (a) Slide size compatibility: While the slide tray design of the TB-Scan system can accommodate most commercial slides, some slides may be too large to fit into the tray slot or too small and prone to falling out of the slide tray. This could impact the system's ability to process certain slide formats effectively; (b) Stain quality: The quality of the manual staining technique can influence the performance of the automated system, as the recognition software relies on color as an important parameter for detecting acid-fast bacilli (AFB). Inconsistent or suboptimal staining may compromise the system's ability to accurately identify AFB. We suggest that commercially available automatic stain systems may well resolve the problems.

In conclusion, manual smear microscopy is the last mile of laboratory automation. We believe such an automated microscope system could achieve higher laboratory testing accuracy and efficiency worldwide and may have the potential to expand to other medical fields, such as pap smears, gram stains, parasite smears, and other smears that require labor-intensive work.

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