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Review Article

A Comprehensive Review of Tuberculosis Detection and Prediction Using Hybrid Machine Learning Models on Ziehl-Neelsen-Stained Microscopy Images

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ABSTRACT

Tuberculosis (TB) remains a critical global health challenge, particularly in resource-constrained settings where timely and accurate diagnosis is essential for effective disease management and control. Traditional diagnostic methods, such as Ziehl-Neelsen (ZN)-stained sputum microscopy, are widely employed for detecting *Mycobacterium tuberculosis*; however, these techniques are inherently subjective and prone to variability due to their reliance on manual interpretation. In response, an increasing body of research has applied deep learning (DL)-based approaches to automate TB detection from microscopy images. This systematic review synthesizes findings from 67 studies that have explored various machine-learning techniques for TB diagnosis using ZN-stained images. A structured literature search was conducted across multiple scientific databases, including PubMed, IEEE Xplore, Scopus, and ScienceDirect. Studies were selected based on their focus on DL applications for TB detection using ZN-stained images. The reviewed methodologies encompass various stages, including image preprocessing, feature extraction, classification strategies, and performance evaluation metrics. Our review reveals that DL models, particularly those employing automated feature extraction and classification, are predominantly used, with some studies reporting accuracies of up to 100%. This review provides a comprehensive overview of state-of-the-art methodologies, including image preprocessing, feature extraction, classification strategies, and performance evaluation metrics. Notably, the evidence indicates that convolutional neural network (CNN)-based

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approaches offer the highest promise due to their robust ability to detect subtle features in stained images. Consequently, future research will focus on developing and optimizing CNN-based models to further enhance TB detection, ultimately improving diagnostic outcomes and supporting more effective TB control strategies.

Keywords: Convolutional neural network, hybrid machine learning, medical image processing, tuberculosis detection, Ziehl-Neelsen staining

INTRODUCTION

TB remains a significant global health challenge, caused by *Mycobacterium tuberculosis*, which usually affects the lungs but can spread to other organs. According to the World Health Organization (WHO), in 2024, Malaysia reported an incidence of 74 new and relapse TB cases per 100,000 population and 3,140 TB-related deaths. These figures underscore the significant public health challenge posed by TB in the country (WHO, 2024). TB is an airborne infectious illness transmitted when people with active pulmonary TB cough, sneeze, talk, sing, or laugh. It mostly affects the lungs, but it may also impact the spine, brain, and kidneys. Although TB transmission requires prolonged and close contact, its latent form (latent tuberculosis infection or LTBI) allows the bacteria to persist within the host for years without causing symptoms, only becoming active when the immune system is compromised (Tobin & Tristram, 2024). However, comprehensive data on LTBI prevalence in Malaysia remains limited. A study by MacLean et al. (2020) using the tuberculin skin test (TST) reported an LTBI prevalence of 68.20% among inmates in Malaysian prisons, indicating significant latent transmission reservoirs. The high burden of TB and LTBI necessitates advancements in diagnostic approaches to ensure timely detection and treatment, particularly in resource-limited settings where conventional methods face significant challenges. Many individuals with active TB may not immediately realize they are infected, as the disease can sometimes develop slowly over weeks or even months without showing noticeable symptoms (Suliman et al., 2019). When symptoms do appear, they often resemble those of common illnesses such as the flu and may include fever, weight loss, persistent cough, and fatigue. Since TB can take up to six weeks to manifest, delayed detection contributes to continued transmission (Nor et al., 2021). Figure 1 illustrates that an individual with active TB can unknowingly transmit the bacteria to a healthy person, underscoring the urgency for improved early detection methods (Cambier et al., 2014).

TB diagnosis traditionally involves multiple laboratory techniques, including chest radiography, computed tomography (CT) scans, and microbiological assessments of sputum samples. Among these, Ziehl-Neelsen ZN staining for sputum smear microscopy remains one of the most widely used diagnostic techniques in resource-limited settings, as it enables direct visualization of acid-fast bacilli (AFB) under a microscope (Ghosh et al., 2022; Surani et al., 2021). Figure 2 displays an image of TB detected using ZN-stained microscopy.

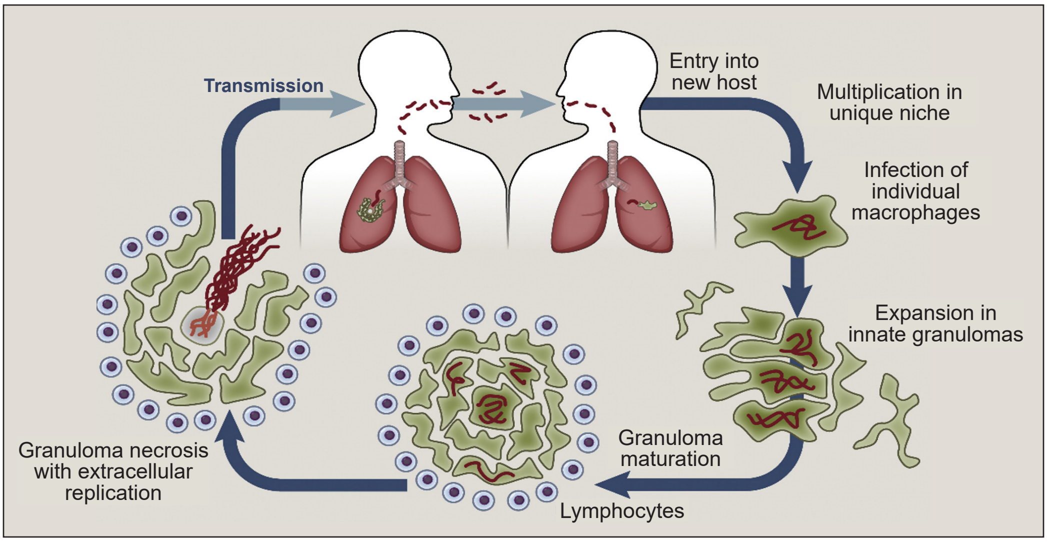

Figure 1. Development of tuberculosis illustration (Cambier et al., 2014)

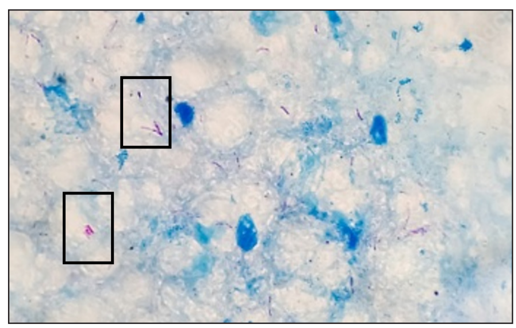

Figure 2. Tuberculosis was detected using Ziehl-Neelsen-stained microscopy under 40× magnification

The image shown below is also displayed. ZN staining is a special bacteriological stain used to identify AFB, which retains the red carbol fuchsin stain even after being washed with acid alcohol. This image is a sample from the Faculty of Medicine laboratory for TB analysis at Universiti Teknologi MARA, Shah Alam. The characteristic appearance of TB bacilli under ZN staining includes curved, rod-shaped bacteria, often wrapped together in cord-like formations. While this staining method provides a direct way to detect TB, it is highly time-consuming since researchers or pathologists must manually examine microscopic fields to locate the tiny bacilli. Given that TB bacteria measure approximately 1-2 micrometers, detecting them requires magnification of at least 40×, making the process labor-intensive and prone to human error. These challenges highlight the urgent need for advanced technologies, like machine learning (ML), to enhance TB diagnosis efficiency and reduce diagnostic errors associated with manual interpretation.

The application of artificial intelligence (AI) and ML in TB detection has shown considerable potential in improving accuracy, efficiency, and automation (Hooda et al., 2017; Lakhani & Sundaram, 2017). CNNs, a class of DL models specialized in image processing, have demonstrated remarkable performance in medical image classification. Studies have validated CNN-based approaches for TB detection using chest X-rays, achieving high diagnostic accuracy and significantly reducing the time required for analysis (Mujeeb Rahman et al., 2025; Nafisah & Muhammad, 2024). Sarawagi et al. (2024) further reinforced these findings, demonstrating the potential of CNN architectures in streamlining TB detection workflows in clinical settings. However, while CNN models have been widely applied to radiographic images, their application to ZN-stained sputum smear microscopy images remains underexplored. ZN-stained images present unique challenges such as non-uniform staining, overlapping bacilli, and background noise, necessitating specialized ML techniques tailored for effective feature extraction and classification.

Traditional TB diagnostic methods, such as ZN-stained microscopy, are prone to human error, time-consuming, and suffer from variability in technician expertise, which can impact the accuracy and reliability of diagnoses. While CNNs have been extensively applied to various medical imaging tasks, their specific application to TB detection using ZN-stained tissue sample microscopy images remains underexplored. Moreover, most ML approaches in TB detection lack emphasis on optimizing pre-trained CNN models, such as VGG16, to effectively address the unique challenges associated with ZN-stained microscopy images, including variations in stain intensity and noise artifacts. This review aims to bridge this gap by systematically analyzing and evaluating ML techniques tailored for ZN-stained TB microscopy images, with a focus on pre-trained CNN models and their optimization strategies.

To address these challenges, ML offers a transformative approach for TB diagnosis by automating and standardizing image-based detection. DL models, particularly CNNs, can analyze microscopy images with high precision, reducing the subjectivity associated with manual analysis. Automated image analysis significantly reduces diagnosis time, enabling rapid screening and early intervention. Additionally, ML models eliminate intra- and inter-observer variability, ensuring consistent and reproducible diagnostic outcomes. AI-driven diagnostic frameworks can be integrated into digital pathology systems, enabling remote diagnostics and supporting telemedicine initiatives in resource-constrained regions. Furthermore, this review provides a comparative analysis of existing ML-based approaches for TB detection in ZN-stained images, highlights their strengths and weaknesses, and discusses potential improvements in model training, feature selection, and generalization performance. By systematically evaluating existing ML approaches and their applicability to microscopy-based TB diagnosis, this study aims to bridge the gap between traditional diagnostic techniques and modern computational advancements, ultimately contributing to improved TB detection and disease management.

Traditional TB Diagnosis Approaches

TB remains a daunting worldwide health concern, despite substantial breakthroughs in diagnostic technologies. From a clinical perspective, TB illness is evaluated using a complete medical assessment that includes a detailed medical history, physical examination, and several types of diagnostic testing (Centers for Disease Control and Prevention [CDC], 2025). These tests typically include tuberculin skin tests (TST) or TB blood tests (interferon-gamma release assays), chest radiography, and laboratory evaluations such as sputum smear microscopy, mycobacterial culture, and molecular tests for drug resistance (Pai et al., 2016). Nevertheless, a definitive diagnosis is based on laboratory procedures that directly detect the presence of tuberculosis germs, most notably sputum smear microscopy and culture. While mycobacterial culture is regarded as the gold standard because of its great sensitivity, it needs an incubation time of up to eight weeks, delaying treatment action (Liang et al., 2022; McClean et al., 2024). Polymerase chain reaction (PCR)-based technologies, such as the GeneXpert MTB/RIF test, provide quick detection and simultaneous drug resistance profiling, but they are restricted by cost and resource restrictions in many endemic areas (Horne et al., 2019).

ZN staining remains an important component of this diagnostic system, especially in resource-limited situations. ZN-stained sputum smear microscopy is commonly used to identify acid-fast bacilli (AFB) associated with *Mycobacterium* TB (Bhandari R, 2021; Masali et al., 2021). The ZN staining method involves several key steps. First, carbol fuchsin is applied to the sputum smear, which penetrates the lipid-rich cell walls of the *Mycobacterium tuberculosis* (TB) bacteria, staining them red. Following this, an acid-alcohol solution is used to decolorize the smear. This step removes the stain from non-acid-fast organisms, leaving only the TB bacteria-stained red. Finally, methylene blue is applied as a counterstain, providing a blue background that contrasts with the red-stained TB bacteria, enhancing their visibility. This causes TB bacteria to appear as brilliant red rods under a light microscope (Bayot et al., 2023; Dzodanu et al., 2019). Figure 3 illustrates the ZN-stained process (LaboratoryInfo, 2022), which is a successful approach that is strongly reliant on the knowledge of the microscopist, resulting in possible inter-observer variability and reduced sensitivity in cases with low bacterial load (Behr et al., 2022; Zaporojan et al., 2024).

Manual examination of ZN-stained smears has been the cornerstone of TB diagnosis in many regions due to its cost-effectiveness and simplicity. However, its accuracy is often compromised by subjective interpretation and inter-observer variability (Perez-Siguas et al., 2023). Despite these limitations, ZN-stained smear microscopy remains critical in high-burden settings that lack advanced diagnostic technologies (Tummalapalli et al., 2024). Automated image analysis systems have been developed to overcome these challenges. These systems employ ML and computer vision techniques to analyze digital images of ZN-stained smears, reducing diagnostic variability, expediting the screening process, and maintaining high sensitivity and specificity (Bhaskar et al., 2023). Additionally, automation facilitates digital archiving and remote consultation, which are valuable for large-scale

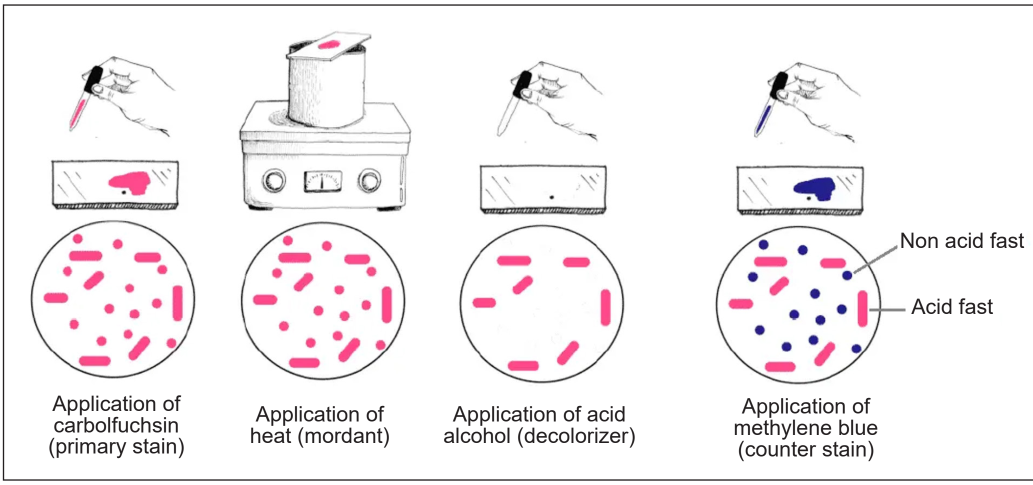

Figure 3. Ziehl-Neelsen-stained process (LaboratoryInfo, 2022)

TB control programs (Shwetha et al., 2021). Although mycobacterial culture and PCR-based assays like ARIMA offer higher sensitivity and rapid drug resistance detection, their high costs and infrastructure requirements limit their widespread use in many endemic areas (Campelo et al., 2021; Li et al., 2022). Therefore, ZN-stained smear microscopy, particularly when enhanced with automated analysis, remains the frontline diagnostic tool for TB screening due to its affordability and rapid turnaround. While previous reviews have broadly examined ML applications in TB detection, most have focused on radiographic images such as chest X-rays or molecular diagnostic methods. However, the application of ML to ZN-stained sputum smear microscopy images remains underexplored. Thus, future research should focus on further integrating automated image analysis with conventional diagnostic workflows to optimize TB control strategies globally.

ML for TB Detection

TB remains a persistent global health challenge, prompting the exploration of innovative diagnostic strategies that extend beyond conventional methods. Traditional techniques such as sputum smear microscopy, mycobacterial culture, and PCR-based assays have been instrumental in TB diagnosis. However, these methods often suffer from limitations. Manual smear microscopy is hindered by subjectivity and inter-observer variability, culture methods are time-consuming, and molecular techniques, though rapid, are expensive and require sophisticated infrastructure (Afsar et al., 2018; Iqbal et al., 2023). In recent years, ML has emerged as a promising tool for improving TB diagnosis. DL techniques, particularly CNN, have revolutionized medical image analysis by automatically learning hierarchical features from complex data. Early research demonstrated the potential of CNN in detecting TB from chest radiographs, with diagnostic accuracies exceeding 90%

(Rajaraman et al., 2022). These encouraging results have spurred further investigation into the use of ML to analyze both radiographic images and ZN-stained sputum smears. However, while most prior reviews primarily focus on ML applications in chest X-ray analysis, limited studies have systematically explored its use in ZN-stained microscopy images, a gap that this review aims to address.

Subsequent studies have expanded on these findings, developed efficient deep network architectures for ZN-stained microscopy images, reporting sensitivities and specificities in the range of 90–95% (Tamura et al., 2024; Witarto et al., 2024). Saini et al. (2023) further refined the approach by integrating segmentation and visualization techniques into CNN frameworks, achieving robust detection accuracies near 93% across varied datasets. These studies illustrate that automated analysis not only improves diagnostic speed but also minimizes human error by providing consistent, objective assessments. Moreover, hybrid approaches combining CNN-based feature extraction with traditional classifiers, such as support vector machines (SVMs) or random forests, have been explored to optimize decision boundaries and further enhance diagnostic performance (Hansun et al., 2023). These methods have shown promise, with several studies reporting accuracies comparable to or even surpassing standalone DL models. Despite these advances, challenges remain. The availability of large, well-annotated datasets is critical for training DL models effectively, and generalizability across diverse populations and imaging conditions is still under investigation (Ahmed et al., 2023). Furthermore, variations in ZN-stained smear characteristics, including stain intensity and background noise, remain a significant hurdle that previous reviews have not extensively analyzed. Addressing these challenges, this study provides a comparative analysis of different CNN architectures, particularly pretrained models like VGG16, and evaluates their suitability for TB detection in ZN-stained microscopy images. Additionally, the review explores optimization strategies for feature extraction and classification, bridging the gap between traditional and AI-driven diagnostic approaches.

Nevertheless, the integration of ML into TB diagnostics represents a significant step forward, with potential applications ranging from automated screening in resource-limited settings to digital archiving and remote consultation for large-scale TB control programs. In summary, the application of machine learning, especially CNNs, has the potential to revolutionize TB detection by offering rapid, accurate, and consistent diagnoses. As ongoing research continues to refine these models and address existing challenges, ML-based approaches are poised to become an integral component of global TB control strategies. This review aims to provide a more targeted analysis of ML applications for ZN-stained images, offering insights into dataset standardization, model generalization, and the real-world feasibility of AI-assisted diagnostics, thereby setting it apart from earlier studies that have focused mainly on radiographic imaging.

Innovations in Medical Diagnostics in Malaysia

The exploration of DL applications in Malaysia has expanded across various fields, particularly in medical diagnostics, agriculture, and engineering, highlighting the growing impact of AI on improving operational efficiency and accuracy. Studies such as Hosain et al. (2024) emphasize the parallels between structured learning approaches and DL model development, particularly in enhancing diagnostic outcomes within the Malaysian healthcare system. Awang et al. (2019) delve into the clinical determinants of severe pulmonary tuberculosis, reinforcing the need for advanced techniques such as CNNs to automate chest radiograph interpretation and improve TB diagnosis. Similarly, Toba et al. (2020) demonstrate that DL models can outperform experienced clinicians in diagnosing congenital heart disease from radiographic images, highlighting the potential of these technologies in improving diagnostic accuracy across various medical conditions in Malaysia. However, most DL studies in Malaysia focus on radiographic imaging (X-rays, CT scans, and magnetic resonance imaging [MRI]), such as Kotei and Thirunavukarasu (2024), while few have addressed the complexity of ZN-stained sputum smear microscopy. Given the unique challenges posed by these images, such as variations in staining intensity and the presence of artifacts, specialized CNN architectures are required to optimize detection performance.

Beyond the healthcare sector, Lu et al. (2020) explore the efficacy of DL methods like LSTMs combined with fully convolutional networks (FCNs) in brain signal processing, underscoring their applicability to other domains, such as neurotechnology and healthcare in Malaysia. Carvalho et al. (2023) further contribute to this narrative by demonstrating how DL models can enhance feature classification accuracy, which could be applied to school health services, ensuring better health outcomes for children in Malaysia. However, unlike large-scale chest radiograph datasets, annotated ZN-stained smear microscopy datasets are scarce in Malaysia. This necessitates innovative approaches such as transfer learning, data augmentation, or synthetic dataset generation to improve model robustness and generalizability across diverse clinical settings.

In medical imaging, Alaskar et al. (2019) show how DL models, such as Alex Net and Google Net, can effectively detect ulcers in wireless capsule endoscopy (WCE) images, offering valuable insights for broader healthcare applications, including TB diagnostics. Xie et al. (2020) present a novel approach using a fully CNNs to detect pulmonary tuberculosis lesions, achieving impressive diagnostic metrics, which are crucial for improving early TB detection in Malaysia. The use of ensemble DL models for TB detection, as discussed by Hwa et al. (2019). It also highlights the importance of leveraging innovative semi-automated methods to differentiate between clinically pulmonary TB and lung cancer, thus contributing to the effective management of TB in high-prevalence regions. However, while CNNs have demonstrated effectiveness in various Malaysian healthcare applications, their direct applicability to TB detection in ZN-stained images remains underexplored. Optimizing pre-trained models such as VGG16 specifically for smear microscopy is crucial to improving sensitivity and specificity in this context.

Finally, Tiwari et al. (2023) extend the application of DL techniques into engineering, specifically in fault diagnosis, emphasizing model robustness through dropout techniques, which is essential for improving diagnostic accuracy across diverse sectors in Malaysia. Collectively, these studies highlight the transformative potential of DL in Malaysia, particularly in enhancing diagnostic accuracy, operational efficiency, and disease management in healthcare, while also contributing to advancements in other critical fields, such as agriculture and engineering. Moreover, AI adoption in Malaysia is still evolving, with regulatory and infrastructural challenges. Discussing how automated TB detection could be integrated into Malaysia's public health policies or screening programs (e.g., leveraging mobile diagnostics for rural areas) enhances the practical relevance of AI-driven approaches. These findings highlight the role of DL technologies in addressing the unique challenges faced by Malaysia, positioning AI as a key tool in enhancing diagnostic performance and public health outcomes.

METHODOLOGY

The analysis includes studies on tuberculosis and machine learning, with a focus on "ZN-stained". The queries returned to several journal and conference publications. This survey solely considers peer-reviewed literature for systematic reviews. Table 1 shows the search engines used, which included Google Scholar, Elsevier, and Springer. The number of search results indicates the number of articles retrieved by search engines for the specified keywords. The number of relevant articles denotes the number of items that passed the initial screening procedure and were judged possibly relevant for a thorough assessment. The bibliographic part of the papers was also examined. The method was iterated until no further items were located.

Prospective research articles are identified, screened, and chosen based on their eligibility. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) model, depicted in Figure 4, outlines the overall publications reviewed in the research. It shows a step-by-step flow chart for the detection technique. Papers that were unrelated to ZN-stained (100 articles) were not considered. After examining 156 publications, 39 were

Table 1
Summary of search results and retrieved relevant articles

Database engines	Number of searches	Number of relevant articles
Elsevier	1,006	20
Springer	217	10
Pubmed	240	26
IEEE Xplore	100	6
MDPI	200	4t
Taylor & Francis	800	10

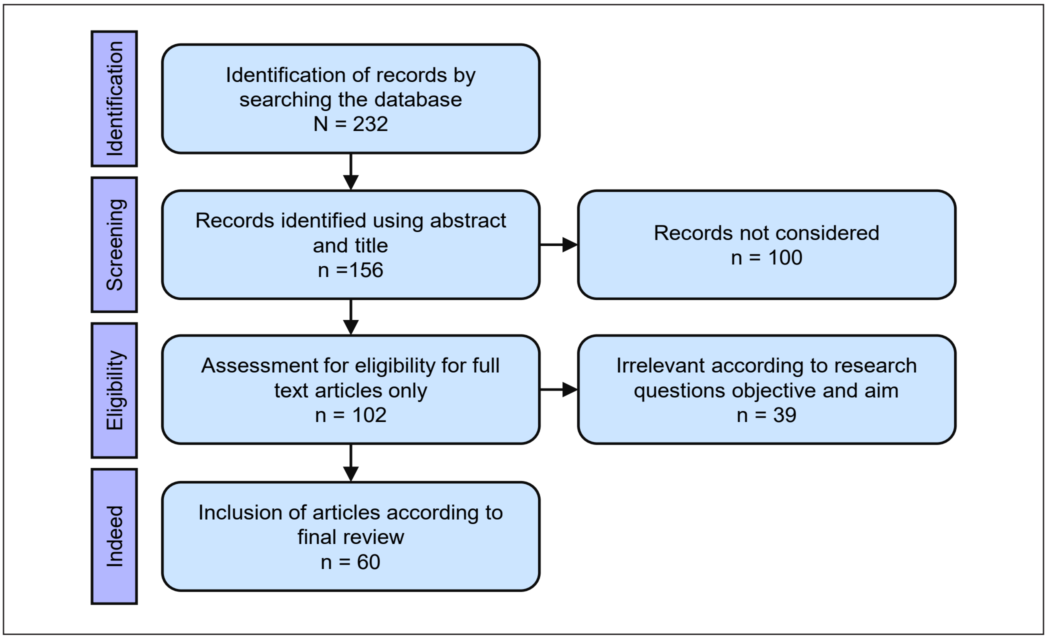

Figure 4. PRISMA model for the depiction of inclusion and exclusion of records

discarded based on title and abstract analysis. The preprint version and duplicate publications were also eliminated. After assessing the quality of published research, 102 papers were selected, with 150 discarded for not being research articles or relevant concepts. As a result, 60 papers were chosen for a detailed assessment.

Tuberculosis Detection and Prediction Framework

A detailed methodology for tuberculosis detection and prediction using a hybrid ML approach is presented in a framework. Figure 5 shows that the framework is structured into four essential phases. In Phase 1, data collection and initial processing occur, during which images of acid-fast bacilli are obtained from the clinical laboratory at Hospital Puncak Alam. Phase 2, referred to as data development, involves refining and transforming the raw dataset. This stage focuses on constructing a well-organized dataset suitable for both training and validating predictive models by applying preprocessing techniques, such as normalization, feature engineering, and data augmentation, to optimize model performance. Phase 3 represents the core of the framework, where the prediction model is trained and subsequently validated. Here, a CNN utilizing the VGG16 architecture is employed. CNNs are particularly adept for this task due to their ability to discern subtle color differences in images, which is a crucial factor, given that the ZN staining method exploits the unique properties of the bacterial cell wall, rich in mycolic acid, rendering it resistant to decolorization by acid-alcohol. This staining technique is critical for diagnosing

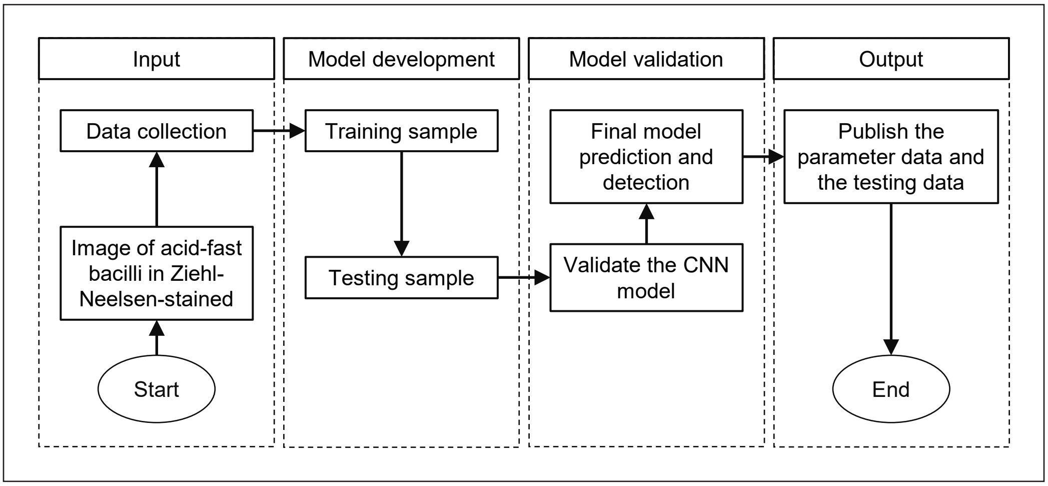

Figure 5. The framework for modelling the tuberculosis detection and prediction on Ziehl-Neelsen-stained slides using hybrid machine learning
Note. CNN = Convolutional neural network; TB = Tuberculosis; ZN = Ziehl-Neelsen

TB in clinical samples like sputum smears or tissue sections. Finally, the CNN model leverages the meticulously curated and preprocessed dataset from Phase 2 to perform accurate TB detection and prediction.

RESULTS AND DISCUSSION

Data Collection

The data for this study were obtained from the Clinical Diagnostic Laboratories (CDL) at Hospital UiTM Puncak Alam. ZN staining is a widely used technique in microbiology and pathology for detecting acid-fast bacilli, particularly *Mycobacterium tuberculosis*. This method capitalizes on the high mycolic acid content in bacterial cell walls, rendering them resistant to acid-alcohol decolorization. Despite its diagnostic importance, analyzing ZN-stained samples remains a labor-intensive process, requiring skilled professionals for accurate interpretation. Figure 6 presents a sample of the dataset used in this study. However, one of the main challenges in working with ZN-stained images is their inherent variability due to differences in staining intensity, image contrast, and sample preparation techniques. Addressing these inconsistencies is crucial

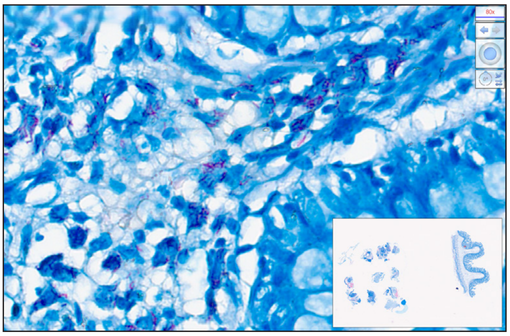

Figure 6. One of the Ziehl-Neelsen-stained microscopy images under 80× magnification using NDP.view2 software

for improving the robustness of automated TB detection models. Since the bacilli are extremely small, measuring approximately 1 μm in size, a magnification of 40× or higher was required to visualize them. As a result, a single glass slide could contain thousands of images, with the bacilli appearing as red-colored rods. However, capturing and saving these images was a time-consuming process, requiring meticulous effort to ensure proper documentation and storage.

Comparison of Detecting TB Using ML

Recent advancements in ML have paved the way for automating TB detection, significantly reducing the burden on healthcare professionals. CNNs have demonstrated exceptional performance in medical image analysis by autonomously learning hierarchical features from complex datasets. Initial studies on chest radiographs reported diagnostic accuracies exceeding 90%, motivating further exploration of ML techniques for TB detection (Lakhani & Sundaram, 2017).

Table 2 summarizes various ML models applied to TB detection. While many studies focus on chest X-rays due to their rapid image acquisition and accessibility, relatively few have explored the application of ML on ZN-stained images. The comparative analysis reveals that most studies have leveraged CNN-based models, such as VGG16, ResNet, and EfficientNet, achieving high accuracy levels. However, a key limitation is that ZN-stained sample analysis requires extensive manual effort, limiting its widespread adoption in automated diagnostic workflows. Despite this, automating ZN-stained smear analysis could provide substantial benefits, particularly in resource-limited settings where access to advanced imaging modalities is constrained.

Among the different ML architectures applied to TB detection, CNN-based models, particularly VGG16, have consistently demonstrated superior performance. The high accuracy of CNNs can be attributed to their ability to automatically extract and learn hierarchical features from image data, reducing reliance on manual feature engineering. However, deeper models such as ResNet and EfficientNet, while offering better feature extraction, tend to require more computational power, making them less suitable for real-time clinical deployment in resource-limited settings. Additionally, studies reporting exceptionally high accuracy (up to 100%) raise concerns about potential overfitting, particularly if models have been trained on small datasets without proper validation. Future work should ensure rigorous evaluation using large-scale, diverse datasets and external validation cohorts to confirm the generalizability of these models.

Challenges in ZN-Stained Image Processing

ZN-stained images present unique challenges for automated analysis compared to chest X-rays. Variability in staining intensity, contrast, and background noise can impact model

Table 2
Automated image detection

Method	Sample	Accuracy (%)	Novelty
ResNet and EfficientNet (Munadi et al., 2020)	Chest X-ray	89.92 and 94.80	Evaluating the impact of three image enhancement techniques (UM, HEF, and CLAHE) on improving the performance of DL models (ResNet and EfficientNet) for tuberculosis detection in chest X-ray images
CNNs (DenseNet201) (Rahman et al., 2020)	Chest X-ray	96.47	Demonstrating that tuberculosis detection accuracy improves when using segmented lung images rather than whole chest X-ray images, with DenseNet201 achieving superior performance
SVM (Hrizi et al., 2022)	Chest X-ray	~93	Optimizing tuberculosis detection by integrating a genetic algorithm (GA) for feature selection with a modified SVM classifier
Image segmentation (Al-Timemy et al., 2021)	Chest X-ray – TB, Covid-19	91.60	Developing a computationally efficient pipeline using ResNet-50 for deep feature extraction and an ensemble of subspace discriminators to accurately distinguish COVID-19, TB, and other chest infections from X-ray images
CNN (Guo et al., 2020)	Chest X-ray	98.46	Integrating CNN modifications, artificial bee colony algorithm-based fine-tuning, and a linear average-based ensemble method to enhance TB detection and localization in CXRs while also differentiating among seven TB-related manifestations using class activation mapping for visual interpretability
Automated image analysis using CNN and traditional ML (Huang et al., 2022)	ZN-stained smear	88–90	Evaluating the μ-Scan 2.0 automation system in a multi-center, double-blind trial, demonstrating its potential to enhance TB smear microscopy sensitivity, efficiency, and remote accessibility for resource-limited areas
CNN (Panicker et al., 2018)	ZN-stained smear	97.13	An automatic TB detection method from sputum smear images using image binarization and CNN-based classification, improving diagnostic speed and accuracy compared to conventional microscopy
CNN-based model (Tasci et al., 2021)	Chest X-ray	97.50	Proposing a voting and preprocessing variations-based ensemble CNN model incorporating Bayesian optimization-weighted soft voting and CLAHE-enhanced image transformations, achieving state-of-the-art accuracy in TB detection from CXR images.
CNN (Rajaraman & Antani, 2020)	Chest X-ray	95	Leveraging modality-specific knowledge transfer from large-scale chest X-ray datasets and integrating a stacked ensemble of top-performing DL models to enhance TB detection accuracy
U-Net segmentation (Rajaraman et al., 2021)	Chest X-ray	~90	Training CXR modality-specific U-Net models for semantic segmentation of TB-consistent findings and enhancing segmentation performance by augmenting training data with weak TB-consistent localizations derived from a DL classifier

Note. CNN = Convolutional neural network; TB = Tuberculosis; ZN = Ziehl-Neelsen; DL = Deep learning; UM = Unsharp masking; HEF = Histogram equalization filtering; CLAHE = Contrast Limited Adaptive Histogram Equalization; SVM = Support vector machine; GA = Genetic algorithm; CXR = Chest X-ray; AI = Artificial intelligence; U-Net = U-shaped convolutional network performance, leading to false positives or negatives (Shah et al., 2017). Traditional image preprocessing techniques such as contrast enhancement, histogram equalization, and denoising can mitigate these challenges to some extent. However, DL-based preprocessing methods, such as generative adversarial networks (GANs) for image enhancement or self-supervised learning for feature extraction, could provide more robust solutions. Another major limitation is the presence of overlapping bacilli in ZN-stained smears, which can confuse segmentation algorithms. Advanced segmentation models, such as U-Net and Mask R-CNN, can be integrated into ML pipelines to enhance bacillus localization and classification accuracy.

Clinical Integration and Real-World Applications

Although CNN-based models have demonstrated high accuracy, their integration into clinical workflows remains a challenge. One major barrier is the lack of model interpretability; clinicians need to understand why a model made a certain prediction. Explainability techniques such as gradient-weighted class activation mapping (Grad-CAM) and SHapley Additive exPlanations (SHAP) could enhance trust in AI-assisted TB diagnosis by visualizing which image regions contributed to the model's decision (Narkhede, 2024). Additionally, regulatory considerations and ethical concerns must be addressed before deploying AI-based TB detection in real-world settings. Ensuring fairness and avoiding biases in AI models is crucial, especially when datasets are skewed towards specific populations or biased by certain staining methods. Implementing human-in-the-loop AI systems, where models assist rather than replace clinicians, could strike a balance between automation and medical expertise.

While CNNs have dominated TB detection research, alternative approaches could further enhance performance. Hybrid models that combine CNNs with traditional ML classifiers, such as SVMs or random forests, have shown promise in improving diagnostic accuracy (Narkhede, 2024). Moreover, transformer-based architectures, such as vision transformers (ViTs), could offer superior feature representation for medical image analysis, though their effectiveness on ZN-stained images remains largely unexplored. Self-supervised learning, where models learn representations from unlabeled data, could also be beneficial, especially given the limited availability of annotated ZN-stained datasets. This approach has the potential to reduce dependency on large, labeled datasets while improving model robustness.

Review of TB Detection on ZN-Stained Using ML

The review of TB detection using ZN-stained microscopy images through ML is comprehensively summarized in Table 3. This review specifically focuses on research studies that have utilized ZN-stained smear images as the primary data source for TB detection, in contrast to the more extensively studied chest X-ray (CXR) images. While CXRs have been widely adopted in TB screening due to their accessibility and ease of acquisition, ZN-stained smear microscopy remains the gold standard for bacteriological confirmation of TB, particularly in low-resource settings. However, manual examination of these smears by trained microbiologists is time-consuming, labor-intensive, and prone to interobserver variability, leading to potential inconsistencies in diagnosis.

The table provides an overview of key research works in this domain, detailing essential aspects such as the author(s), the type of sample used (i.e., ZN-stained sputum smear images), the ML techniques and architectures employed, including CNNs, U-Net, hybrid DL models, and other automated classification methods—and the reported performance metrics, such as accuracy, sensitivity, and specificity. One of the primary challenges associated with analyzing ZN-stained microscopy images stems from their inherent characteristics, including low contrast, background noise, staining inconsistencies, and the presence of artifacts, all of which can hinder the performance of traditional image-processing approaches. Despite these challenges, the reviewed studies demonstrate that well-designed machine-learning pipelines can achieve promising diagnostic accuracy, typically within the range of 88 to 90%.

These findings suggest that automated TB detection from ZN-stained smears has significant potential for clinical applications, particularly as a supportive tool to aid pathologists and laboratory technicians in making diagnostic decisions. By leveraging DL models trained on large-scale annotated datasets, such systems can enhance diagnostic efficiency, reduce human error, and improve consistency across different laboratory settings. Furthermore, the integration of ML-based TB detection into routine clinical workflows could be particularly transformative in resource-limited regions where access to skilled personnel and advanced diagnostic tools remains a significant barrier to timely and accurate TB diagnosis. Table 3 presents a summary of relevant studies, highlighting their methodologies, datasets, and outcomes, thereby providing a comparative analysis of the current state in this field. This review underscores the growing importance of AI in infectious disease diagnostics and reinforces the notion that automated image-based TB detection could serve as a valuable adjunct to existing diagnostic methods, ultimately contributing to global TB control efforts.

In summary, our review indicates that among the various ML models applied to TB detection using ZN-stained images, CNN-based approaches have demonstrated the highest performance. Some studies even report an accuracy of up to 100%. This remarkable level of accuracy not only exceeds that of traditional ML methods and hybrid models but also highlights the robustness of CNN in automatically extracting and processing complex image features. Such outstanding performance provides a strong rationale for focusing our research on CNN-based models, as they offer a reliable and efficient solution for automated TB diagnosis.

Table 3
Tuberculosis detection on Ziehl-Neelsen-stained slides using machine learning

Method / Technique	Samples	Accuracy (%)	Novelty
CNN - VGG16, ResNet50, and SqueezeNet (Shwetha et al., 2021)	200 images	97	The usage of SqueezeNet for bacilli detection, which achieves 97% accuracy with a lightweight model, is more efficient than VGG16 and ResNet50 while maintaining excellent performance
Coarse (RGB) and fine (Sauvola) level segmentation (Samuel & Baskaran, 2021)	Not described	98.70	Integration of RGB thresholding and Sauvola's adaptive thresholding for TB bacilli segmentation, combined with shape descriptors for precise feature extraction, enables an automated and efficient TB detection system that reduces manual effort while improving sensitivity and specificity
Pat-Scan, scanner, and software (Sua et al., 2021)	2,000 images	99	Developing a digital pathology program for detecting and quantifying both typical and atypical mycobacteria in paraffin-embedded ZN-stained tissues
HSV color space transformation and image segmentation (Riza et al., 2022)	51 images	78.68	A freely available, diverse dataset supporting autofocusing, auto stitching, and bacilli segmentation, which enhances algorithm development and validation for automated TB detection
CNN (Zaizen et al., 2022)	Not described	98	Detecting AFB in bronchoscopy samples demonstrates significantly higher sensitivity (86%) compared to conventional bacteriological tests (29%) for the TB diagnosis
CNN - AlexNet, VGGNet-19, ResNet-18, DenseNet, GoogLeNet-incept-v3, In-ceptionResNet-v2, and the classic three-layer model (Shelomentseva & Chentsov, 2021)	Not described	99	A simple three-layer convolutional neural network outperforms advanced transfer learning models like DenseNet and InceptionResNet-v2 for TB bacilli detection in ZN-stained images, highlighting the potential of lightweight CNNs for automated diagnosis
CNN - ResNet-18, ResNet-50, and VGG-16 (Rachmad, 2024)	5,100 images	93.42	Demonstrating that AlexNet outperforms ResNet-18, ResNet-50, and VGG-16 in both accuracy (93.42%) and processing speed (5 min 52 s) for TB detection
CNN (Yang et al., 2020)	21,504 images	87.62	Developing an ML pipeline that combines two CNN models with an active learning framework and logistic regression, achieving high sensitivity (87.13%) and specificity (87.62%) for AFB detection
RegNetX4 (Zurac et al., 2022)	510 images	98.33	Developing a high-performance AI-based mycobacteria identification method using a large dataset of over 260,000 positive and 700 million negative patches from 510 ZN-stained whole slide images

Table 3 *(continue)*

Method / Technique	Samples	Accuracy (%)	Novelty
CNN and SVM (Rachmad et al., 2020)	1,000 images	97.60, 97.90	ResNet-101 architecture combined with SVM for TB bacteria classification in ZN-stained images
CNN – VGG16 (Author's ongoing work)	~1,000 images	-	Developing a hybrid DL model for tuberculosis detection using ZN-stained microscopy images, integrating CNN architectures (such as VGG16) while also exploring preprocessing and segmentation techniques to enhance detection performance

Note. CNN = Convolutional neural network; TB = Tuberculosis; ZN = Ziehl-Neelsen; RGB = Red Green Blue; AFB = Acid-fast bacilli; HSV = Hue Saturation Value; SVM = Support vector machine; DL = Deep learning; ML = Machine learning; Pat-Scan = Pathology scanner; Grad-CAM = Gradient-weighted class activation mapping; SHAP = SHapley Additive exPlanations: RESNET = Residual network; VGG = Visual Geometry Group; DenseNet = Densely connected convolutional network

Despite significant advancements in ML for TB detection, several challenges remain. First, dataset biases, particularly in staining methods and image acquisition settings, can affect model performance across different institutions. Future research should focus on developing standardized datasets that encompass a diverse range of staining variations to improve model generalization. Second, the lack of large-scale, publicly available ZN-stained image datasets hampers progress in this field. Establishing collaborative research initiatives to share anonymized datasets could facilitate better benchmarking and validation of ML models. Federated learning, where models are trained across multiple hospitals without sharing raw data, could be a promising approach to overcome privacy concerns while improving model robustness. Lastly, real-time deployment of ML models in clinical settings requires lightweight architectures that balance accuracy with computational efficiency. Future research should explore model compression techniques such as knowledge distillation or quantization to make CNN-based models more suitable for deployment in resource-limited environments.

CONCLUSION

This comprehensive analysis researched 67 studies relating to machine learning-based TB identification with ZN-stained microscope images. The results show that CNNs are the most successful strategy, consistently outperforming classic ML models in feature extraction and classification. The capacity of CNNs to identify minute patterns in microscope pictures makes them an effective tool for automated tuberculosis diagnosis. Furthermore, this paper emphasizes the importance of hybrid models, which combine CNNs with classical classifiers such as SVMs or VGG16, demonstrating the potential for future performance gains. The study also highlights the importance of image preprocessing methods, such as contrast enhancement and segmentation, in improving model accuracy. Compared to prior reviews, this paper presents a more targeted investigation of ML algorithms applied to ZN-stained microscopy pictures, shedding light on the strengths and limits of various approaches.

Despite these developments, significant hurdles remain to establishing scalable and clinically effective AI models for tuberculosis diagnosis. Future research should focus on increasing dataset diversity to ensure models generalize across varied imaging conditions and patient populations. Hybrid and explainable AI technologies can help to improve model interpretability and clinician trust. Furthermore, real-world clinical validation and deployment methodologies must be investigated to integrate AI-based TB diagnosis into regular diagnostics. Addressing these problems will be crucial in enhancing the role of ML in TB screening, ultimately leading to more accurate and efficient disease diagnosis in global healthcare settings.

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