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Evaluation of Centrifugation Using 4% NaOH for Acid-Fast Bacilli Detection and Ziehl–Neelsen Smear Quality in Sputum Specimens with Negative Rapid Molecular Test Results

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Abstract: This study aimed to evaluate the effect of centrifugation with 4% sodium hydroxide (NaOH) on acid-fast bacilli (AFB) detection using the Ziehl–Neelsen method in sputum specimens with negative Molecular Rapid Test (MRT) results. This laboratory-based experimental study employed a paired design involving 50 sputum specimens with negative MRT results. Each specimen was examined using the Ziehl–Neelsen method before and after centrifugation with 4% NaOH. The primary outcome was the change in AFB detection results, while smear appearance was descriptively assessed based on background clarity and the presence of debris. All specimens (50/50; 100%) remained AFB-negative both before and after centrifugation, indicating that centrifugation with 4% NaOH did not alter AFB detection outcomes. Descriptively, centrifuged smears showed cleaner backgrounds and less debris, which facilitated microscopic examination; however, smear quality was not evaluated using a quantitative scoring system. Therefore, centrifugation with 4% NaOH did not improve AFB detection by the Ziehl–Neelsen method in sputum specimens with negative MRT results. Nevertheless, centrifuged smears appeared visually cleaner during microscopic examination, suggesting a potential benefit for specimen preparation that requires further validation using standardized quantitative assessment.
Keywords: Acid-fast bacilli; centrifugation; Molecular Rapid Test; 4% NaOH; Ziehl–Neelsen

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INTRODUCTION

Tuberculosis (TB) is a communicable infectious disease caused by *Mycobacterium tuberculosis* and remains a major cause of morbidity and mortality from infectious diseases worldwide. TB is primarily transmitted through airborne particles released by individuals with pulmonary TB during coughing, sneezing, speaking, or other respiratory activities (Anggraeni & Rahayu, 2019; WHO, 2025). Despite substantial global control efforts, TB continues to pose a major public health challenge because of its persistently high disease burden, the emergence of drug-resistant strains, and delays in diagnosis and treatment, which may contribute to ongoing transmission within communities (WHO, 2021; WHO, 2025). In 2024, an estimated 10.7 million people developed TB globally, highlighting the continuing magnitude of the disease despite advances in prevention, diagnosis, and treatment (WHO, 2025).

Indonesia is among the countries with the highest TB burden worldwide. According to the WHO Global Tuberculosis Report 2025, Indonesia accounted for approximately 10% of estimated global incident TB cases in 2024, second only to India (WHO, 2025). Rapid and accurate diagnosis is therefore essential to facilitate timely initiation of appropriate treatment, improve treatment outcomes, and interrupt transmission. Laboratory-based diagnosis plays a critical role in achieving these objectives and supporting Indonesia's national tuberculosis elimination program

(Kementerian Kesehatan Republik Indonesia, 2023). Improving access to sensitive and reliable bacteriological testing is particularly important in high-burden settings, where diagnostic delays may sustain community transmission.

Ziehl–Neelsen (ZN) staining for acid-fast bacilli (AFB) remains widely used because it is relatively simple, rapid, and inexpensive. However, its major limitation is its relatively low sensitivity, particularly in specimens containing low numbers of bacilli (Isbaniah et al., 2021). The diagnostic yield of direct smear microscopy depends substantially on bacillary concentration and specimen quality; consequently, smear-negative results may occur when the number of organisms is below the microscopic detection threshold (Steingart et al., 2006). Molecular Rapid Testing (MRT), including assays such as Xpert MTB/RIF, provides greater sensitivity than conventional smear microscopy and is recommended as an initial diagnostic approach for individuals with signs and symptoms of TB (Anwar et al., 2023; WHO, 2024). Nevertheless, ZN microscopy continues to have practical value in certain laboratory settings, particularly where molecular diagnostic capacity is limited and for selected bacteriological monitoring purposes. Therefore, optimization of sputum preparation remains relevant for improving microscopic readability and maximizing the diagnostic yield of smear examination without overstating its performance relative to molecular methods.

One approach to optimizing sputum preparation is chemical homogenization followed by centrifugation. Sodium hydroxide (NaOH) functions as a decontaminating and homogenizing agent by digesting mucoprotein-rich material in sputum, thereby reducing mucus and facilitating separation of the sediment during centrifugation (Safwan et al., 2020). The resulting sediment may contain a higher concentration of cellular and bacterial material, while removal of mucus and nonspecific debris can produce a cleaner microscopic background and facilitate AFB identification. A systematic review of sputum-processing methods reported that chemical processing followed by centrifugation can improve the sensitivity of smear microscopy compared with direct, unconcentrated smears, although the magnitude of improvement varies according to the processing protocol and specimen characteristics (Steingart et al., 2006). Similarly, decontamination–concentration procedures incorporating NaOH have been shown to increase AFB recovery in microscopy compared with direct smear preparation in selected study populations (Ganoza et al., 2008).

In the present study, a 4% NaOH solution was used for sputum homogenization and decontamination before centrifugation. Previous studies have suggested that NaOH treatment can facilitate the breakdown of sputum components and reduce contaminating material, thereby improving preparation of the sediment for microscopic examination (Astuti et al., 2020; Safwan et al., 2020). However, the effectiveness of NaOH depends on several procedural factors, including concentration, duration of exposure, and subsequent processing conditions; excessive chemical exposure may adversely affect mycobacterial recovery. Therefore, the potential benefit of 4% NaOH should be interpreted in relation to the complete specimen-processing protocol rather than assuming that this concentration is universally optimal. This consideration is important because both chemical decontamination and centrifugation can influence the number and distribution of bacilli ultimately deposited on the smear.

Previous studies have reported that sputum concentration by centrifugation may improve microscopic smear preparation and, under some conditions, increase AFB detection (Rambi et al., 2018; Astuti et al., 2020; Steingart et al., 2006; Ganoza et al., 2008). However, evidence specifically addressing centrifugation with 4% NaOH in sputum specimens that yield negative Molecular Rapid Test results remains limited. Importantly, a negative molecular test does not necessarily indicate that a specimen is

paucibacillary; rather, it indicates that *M. tuberculosis* was not detected by the molecular assay under the conditions of testing. Accordingly, whether additional homogenization and concentration can reveal AFB that were not detected by the initial molecular examination remains uncertain and requires empirical evaluation.

The research gap addressed in this study is therefore the limited evidence regarding whether 4% NaOH-assisted homogenization and centrifugation can alter ZN microscopy results and improve the microscopic appearance of smears prepared from MRT-negative sputum specimens. The novelty of this study lies in evaluating both outcomes within the same paired specimens, thereby distinguishing changes in AFB detection from changes in visual smear characteristics. Accordingly, this study aimed to determine the effect of centrifugation with 4% NaOH on Ziehl–Neelsen AFB examination results in sputum specimens with negative MRT results and to descriptively evaluate changes in microscopic smear appearance. The findings are expected to provide evidence for laboratories considering centrifugation as an additional sputum-processing procedure, particularly in settings where conventional microscopy remains part of routine laboratory practice.

METHOD

This study employed a quantitative laboratory experimental approach to evaluate the effect of centrifugation using a 4% sodium hydroxide (NaOH) solution on acid-fast bacilli (AFB) detection by the Ziehl–Neelsen method in sputum specimens with negative Molecular Rapid Test (MRT) results. The study was conducted at the Medan Public Health Laboratory from July 2 to August 2, 2025. A paired experimental design was used, in which each sputum specimen served as its own comparison and was examined before and after centrifugation with 4% NaOH. All study procedures were performed in accordance with the Standard Operating Procedures (SOPs) of the Medan Public Health Laboratory. The reporting of the study was guided by relevant principles of the Standards for Reporting Diagnostic Accuracy Studies (STARD), where applicable to the characteristics of this laboratory-based investigation.

The study population comprised all sputum specimens from patients suspected of having tuberculosis who underwent laboratory testing at the Medan Public Health Laboratory during the study period. Specimens were selected using purposive sampling according to the predefined inclusion and exclusion criteria. A total of 50 sputum specimens with negative MRT results met the study criteria and were included in the analysis. All specimens were processed immediately after receipt at the laboratory in accordance with the applicable SOPs; therefore, no specimen storage was required before testing.

The laboratory equipment included the GeneXpert® System for MRT, sterile single-use sputum containers, a Class II Type A2 Biological Safety Cabinet (BSC), a light microscope, a refrigerated microcentrifuge with a maximum speed of 5,000 rpm, transfer pipettes, staining racks, microscope slides, wooden slide holders, forceps, personal protective equipment, and other standard laboratory glassware. The study materials consisted of 50 sputum specimens with negative MRT results, three specimens classified as “Very Low” by MRT that were used as positive controls for monitoring Ziehl–Neelsen staining quality, GeneXpert® MTB/RIF cartridges, 4% NaOH solution, distilled water, and Ziehl–Neelsen staining reagents consisting of 1% carbol fuchsin, 3% acid alcohol, and 0.1% methylene blue.

The experimental procedure began with examination of sputum specimens using the GeneXpert® Molecular Rapid Test to identify specimens with negative results that fulfilled the study criteria. A 4% NaOH solution was then used for sputum

homogenization and decontamination. Each eligible specimen underwent two Ziehl–Neelsen examinations. First, a direct smear was prepared and examined without centrifugation. Second, the same specimen was subjected to homogenization and decontamination with 4% NaOH followed by centrifugation, after which the resulting sediment was used to prepare a second smear. Both smears were stained using the Ziehl–Neelsen method in accordance with laboratory SOPs. The stained smears were examined under a light microscope using a 100× oil-immersion objective, with at least 100 microscopic fields evaluated for each smear. The results were interpreted according to the International Union Against Tuberculosis and Lung Disease (IUATLD) guidelines (Husna & Dewi, 2020).

The primary outcome was the paired AFB detection result obtained before and after centrifugation with 4% NaOH. In addition, the visual characteristics of the smears were assessed descriptively during microscopic examination, focusing on background clarity and the presence of mucus or debris. Because smear quality was not evaluated using a validated quantitative scoring system and no interobserver agreement assessment was performed, these observations were treated as descriptive supporting findings only. They were not used to infer a quantitative improvement in smear quality or diagnostic sensitivity.

Data were analyzed descriptively using univariate analysis and presented as frequency distributions, percentages, and documentation of microscopic findings. Pre- and post-centrifugation AFB results from the same specimen were compared on a paired basis to determine whether centrifugation produced any change in detection status. The Ziehl–Neelsen results were also compared with MRT findings, which were used as the reference test for describing agreement among specimens that had already been classified as MRT-negative. Because the study population included only MRT-negative specimens, the analysis was not designed to provide a complete estimate of diagnostic sensitivity across both positive and negative tuberculosis cases.

RESULTS AND DISCUSSION

Sample Characteristics

This study included 50 sputum specimens with negative Molecular Rapid Test (MRT) results obtained from the Medan Public Health Laboratory. Each specimen was examined using Ziehl–Neelsen (ZN) staining before and after centrifugation with 4% NaOH. Patient characteristics included age and sex, while specimen characteristics included sputum consistency and color. These variables were documented to describe the study population and the pre-analytical characteristics of the specimens.

Table 1. Characteristics of respondents

Category	Frequency (n = 50)	Percentage
Patient Age		
< 40 years	12	24%
40–60 years	25	50%
> 60 years	13	26%
Patient Sex		
Male	30	60%
Female	20	40%
Total	**50**	**100%**

As shown in Table 1, half of the participants were aged 40–60 years (50%), followed by those aged >60 years (26%) and <40 years (24%). Men accounted for 60%

of the study population, whereas women accounted for 40%. This distribution is broadly consistent with the epidemiological profile of tuberculosis in Indonesia, where TB notifications are generally higher among men and economically productive age groups. Differences in TB occurrence between sexes may be influenced by a combination of biological, behavioral, occupational, and healthcare-seeking factors, including smoking, occupational exposure, mobility, and delays in accessing health services (Kementerian Kesehatan Republik Indonesia, 2023).

The age and sex distributions should, however, be interpreted independently because the present study did not perform a cross-tabulation of age by sex. Therefore, although men constituted the majority of the participants and individuals aged 40–60 years represented the largest age category, the data do not establish that most participants aged 40–60 years were male.

Table 2. Characteristics of sputum specimens

Category	Frequency (n = 50)	Percentage
Specimen Consistency		
Thick	31	62%
Watery	19	38%
Specimen Color		
Yellowish white	13	26%
Clear white	24	48%
Cloudy green	5	10%
Deep yellow	4	8%
Reddish brown	4	8%

Most sputum specimens were thick in consistency (62%), whereas 38% were watery. The most frequently observed color was clear white (48%), followed by yellowish white (26%), cloudy green (10%), deep yellow (8%), and reddish brown (8%). Sputum characteristics are important during the pre-analytical phase because the quality and representativeness of a respiratory specimen may influence the probability of detecting *Mycobacterium tuberculosis*. Specimens originating from the lower respiratory tract are generally more suitable for bacteriological examination than specimens consisting predominantly of saliva.

Sputum quality has been recognized as an important determinant of laboratory performance in both microscopic and molecular TB testing. Roswidianah et al. (2025) emphasized that specimen characteristics can influence diagnostic findings and should therefore be considered when interpreting differences between microscopy and molecular methods. In the present study, the relatively similar distribution of sputum characteristics may have reduced some pre-analytical variation among specimens. Nevertheless, clinical factors that could influence bacillary burden, such as disease severity, radiological findings, treatment history, immune status, or duration of symptoms, were not evaluated. Consequently, the possible contribution of these factors to the negative microbiological findings cannot be excluded.

Acid-Fast Bacilli Examination Before and After Centrifugation

The results of acid-fast bacilli (AFB) examination using Ziehl–Neelsen staining before and after centrifugation with 4% NaOH are presented in Table 3.

Table 3. AFB examination results under two treatment conditions

Category	Negative	Percentage (%)
Without centrifugation	50	100
With centrifugation	50	100

All 50 sputum specimens remained AFB-negative by Ziehl–Neelsen staining both before and after centrifugation with 4% NaOH. Thus, no additional AFB-positive specimens were identified following the concentration procedure. Under the conditions used in this study, centrifugation with 4% NaOH did not improve AFB detection among specimens that had previously tested negative by MRT.

This finding can be partly explained by the analytical limitations of conventional smear microscopy. Ziehl–Neelsen microscopy requires a relatively high concentration of bacilli for reliable detection. The World Health Organization reports that sputum smear microscopy has an approximate analytical limit of detection of 5,000–10,000 bacilli/mL of sputum. WHO therefore considers smear microscopy less sensitive than WHO-recommended rapid molecular diagnostic tests and recommends rapid molecular methods as initial diagnostic tests for pulmonary TB whenever they are available (WHO, 2025).

Evidence from Indonesia similarly demonstrates the difference in diagnostic sensitivity between microscopy and molecular testing. Karuniawati et al. (2023), in a multicenter study conducted in seven Indonesian hospitals, reported that the sensitivity of AFB smear microscopy was significantly lower than that of Xpert MTB/RIF when both methods were evaluated against *M. tuberculosis* culture. Their findings support the interpretation that specimens with low bacillary concentrations may remain smear-negative even when more sensitive diagnostic approaches are used.

However, a negative MRT result should not automatically be interpreted as evidence of paucibacillary tuberculosis or as confirmation that viable *M. tuberculosis* is present at a low concentration. A negative molecular result indicates that the target was not detected by the assay under the conditions of testing. The specimen may contain bacilli below the assay's limit of detection, may truly contain no *M. tuberculosis*, or may be affected by other pre-analytical or analytical factors. Therefore, the absence of AFB in the present study should be interpreted cautiously rather than being attributed exclusively to a low bacillary load.

During microscopic examination, the centrifuged preparations appeared to have cleaner backgrounds than the direct smears. The reduction in visible mucus and debris facilitated observation of the microscopic field. This observation is consistent with the principle of chemical digestion and concentration of sputum, whereby mucus and nonspecific organic material are reduced while particulate material is concentrated in the sediment used for smear preparation.

Previous evidence suggests that specimen-processing methods can improve the performance of smear microscopy under certain conditions. In a systematic review, Steingart et al. (2006) found that chemical processing followed by centrifugation could increase the sensitivity of sputum smear microscopy compared with direct microscopy, although the magnitude of improvement differed substantially among studies. The authors also reported considerable heterogeneity in chemical agents, centrifugation conditions, laboratory procedures, and patient populations. Therefore, the benefit of concentration procedures cannot be assumed to be uniform across laboratory settings.

The effectiveness of centrifugation is also influenced by relative centrifugal force and processing time. Perera & Arachchi (1999) demonstrated experimentally that the recovery of *M. tuberculosis* from processed sputum differed according to centrifugation

conditions and identified 4,000 × g for 15 min as an effective combination under their study conditions. This finding highlights the importance of reporting centrifugation in relative centrifugal force (×g), rather than only revolutions per minute (rpm), because actual centrifugal force varies according to rotor radius.

Chemical processing may also affect bacillary recovery. NaOH is commonly used to liquefy and decontaminate sputum because it digests organic material and suppresses contaminating microorganisms. However, decontamination procedures require careful control because excessive exposure to alkaline agents can adversely affect mycobacterial recovery. Accordingly, NaOH concentration, exposure time, neutralization procedures, centrifugation force, centrifugation duration, and sediment recovery may all influence the final number of bacilli available for microscopic examination.

These considerations may help explain why the cleaner microscopic appearance observed after centrifugation did not result in additional AFB-positive specimens. If *M. tuberculosis* was absent or present at a concentration substantially below the analytical detection threshold of Ziehl–Neelsen microscopy, concentration of the sediment may still have been insufficient to produce a positive smear. Moreover, the effectiveness of a concentration procedure is inherently dependent on the initial bacillary burden in the specimen.

The present findings are consistent with Safwan et al. (2020), who described the role of NaOH in homogenizing sputum and reducing organic contaminants, thereby facilitating microscopic examination. Astuti et al. (2020) similarly reported that the effectiveness of concentration procedures is influenced by the initial concentration of *M. tuberculosis*. Datta et al. (2024) further highlighted the limited sensitivity of conventional Ziehl–Neelsen microscopy in specimens containing low bacterial concentrations.

It is therefore important to distinguish microscopic smear quality from diagnostic sensitivity. A cleaner background can improve readability and reduce interference from mucus and debris, but it does not necessarily increase the concentration of bacilli above the analytical detection threshold. In the present study, the visual appearance of the centrifuged smears suggested a potential improvement in microscopic readability; however, smear quality was not assessed using a validated quantitative scoring system or independent observers. Consequently, this observation should be regarded as descriptive rather than as objective evidence of improved smear quality or diagnostic sensitivity.

Overall, centrifugation with 4% NaOH did not alter AFB detection in MRT-negative specimens. Its potential advantage in the present study appears to be related primarily to specimen preparation and microscopic readability rather than to an increase in diagnostic yield.

Table 4. Changes in AFB Examination Results Before and After Centrifugation with 4% NaOH

Result Before Centrifugation	Negative After Centrifugation	Positive After Centrifugation	Total
Negative	50	0	50
Positive	0	0	0
Total	**50**	**0**	**50**

Table 4 confirms that no change occurred in the paired AFB results. All specimens that were negative before centrifugation remained negative after

centrifugation, and no negative-to-positive or positive-to-negative conversion was observed. Because all paired observations were identical, the dataset contained no discordant pairs on which a statistical test of change could meaningfully operate. Therefore, the finding is most appropriately reported descriptively as complete agreement in AFB status before and after centrifugation.

Differences in Microscopic Appearance Following Ziehl–Neelsen Staining

Differences in microscopic appearance before and after centrifugation with 4% NaOH are shown in Figure 1.

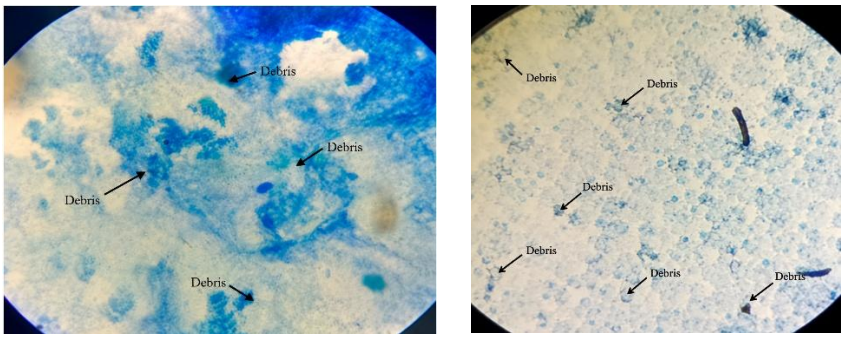

Figure 1. Ziehl–Neelsen staining at 100× magnification: (a) before centrifugation; (b) after centrifugation

Microscopic observation showed that smears prepared without centrifugation had relatively dense backgrounds containing mucus, epithelial cells, leukocytes, and other debris. These components made the microscopic field appear less clear. In contrast, preparations obtained after centrifugation visually showed cleaner backgrounds with less interfering material, allowing structures within the field to be observed more readily. AFB were not identified in either preparation, consistent with the microbiological results obtained for all specimens.

The difference in microscopic appearance is biologically plausible given the function of NaOH and centrifugation during sputum preparation. NaOH facilitates liquefaction and homogenization by disrupting mucous material, while centrifugation separates sedimented particulate material from part of the soluble and nonspecific material remaining in the supernatant. Consequently, smears prepared from the sediment may contain less mucus and background debris than direct sputum smears.

This finding is consistent with Safwan et al. (2020), who reported that sputum-processing procedures involving NaOH can produce cleaner preparations by reducing interfering organic material. Astuti et al. (2020) also reported more homogeneous material following centrifugation, which may facilitate microscopic examination. More broadly, the systematic review by Steingart et al. (2006) demonstrated that processing and concentration techniques can improve smear performance in some settings, although results varied substantially according to the procedure used.

The variability reported among previous studies may partly reflect differences in centrifugation protocols. Recovery of mycobacteria depends not only on whether centrifugation is performed but also on the centrifugal force and duration. Perera & Arachchi (1999) demonstrated that these parameters affect the recovery of *M. tuberculosis* from sputum. Thus, standardization of relative centrifugal force, centrifugation time, sediment volume, and smear preparation is necessary before comparisons can be made reliably across studies.

Despite the apparently cleaner background observed after centrifugation in the present study, no improvement in AFB detection was demonstrated. This finding

emphasizes the distinction between improved microscopic readability and improved diagnostic performance. Removal of mucus and debris may make a smear easier to examine, but it does not necessarily result in a sufficient increase in bacillary concentration to overcome the detection threshold of Ziehl–Neelsen microscopy.

The WHO continues to describe sputum smear microscopy as an insensitive bacteriological test, with an approximate limit of detection of 5,000–10,000 bacilli/mL, and recommends WHO-recommended rapid diagnostic tests that directly detect the *M. tuberculosis* complex as initial diagnostic methods (WHO, 2025). Molecular tests can therefore detect TB at bacterial concentrations at which conventional microscopy may remain negative. This difference in analytical sensitivity provides an important context for interpreting the absence of AFB in specimens that were already MRT-negative.

The present observations should nevertheless be interpreted conservatively because smear quality was not quantified. No validated scoring system was used to assess background clarity, smear thickness, staining uniformity, debris density, or microscopic readability, and the slides were not independently assessed by multiple microscopists. Accordingly, the apparent reduction in background material cannot be considered statistically demonstrated improvement in smear quality.

Future studies should assess smear quality independently from AFB positivity using standardized scoring criteria for background clarity, debris, smear uniformity, staining quality, and microscopic readability, preferably with blinded evaluation by multiple observers to reduce subjectivity. In addition, the practical implications of centrifugation should be considered, including processing time, cost, workload, equipment requirements, and biosafety, as the potential diagnostic benefits of sputum-processing methods must be balanced against increased laboratory complexity (Steingart et al., 2006).

Diagnostic Agreement Between Ziehl–Neelsen Microscopy and Molecular Rapid Testing

The relationship between Ziehl–Neelsen microscopy and MRT results is presented in Table 5.

Table 5. Diagnostic evaluation of the ziehl–neelsen method against molecular rapid test results

Parameter	Value
True Positive (TP)	0
False Positive (FP)	0
False Negative (FN)	0
True Negative (TN)	50
Sensitivity	Not estimable*
Specificity	100%

Note: Sensitivity could not be estimated because no MRT-positive specimens were included (TP = 0 and FN = 0).

All 50 specimens were negative by both Ziehl–Neelsen microscopy and MRT. Within the structure of the present dataset, these observations were categorized as true negatives. No positive or discordant findings were observed. Consequently, sensitivity could not be estimated because the denominator required for its calculation, TP + FN, was zero.

Although the numerical calculation yields a specificity of 100%, this value should be interpreted with considerable caution. The study was deliberately restricted to

specimens with negative MRT results, meaning that the reference-result spectrum did not contain any positive specimens. The resulting value therefore primarily represents complete negative agreement within the selected study sample rather than a comprehensive estimate of the diagnostic specificity of Ziehl–Neelsen microscopy in a general population of individuals being evaluated for tuberculosis.

This distinction is important because valid diagnostic-accuracy assessment requires an appropriate spectrum of participants with and without the target condition. A dataset containing only reference-test-negative specimens cannot determine sensitivity and provides very limited information regarding overall diagnostic performance. Consequently, the principal inference from the present study is not that Ziehl–Neelsen microscopy has 100% specificity, but rather that no additional AFB-positive results were obtained after centrifugation among 50 MRT-negative specimens.

The terminology used for the reference method also requires careful consideration. WHO identifies culture, particularly using liquid media followed by species confirmation, as the conventional reference standard for bacteriological confirmation of TB, whereas WHO-recommended rapid molecular diagnostic tests are currently recommended as initial diagnostic tests because they provide substantially faster results and greater sensitivity than conventional smear microscopy (WHO, 2025). Therefore, in the context of the present study, MRT is more appropriately described as the reference test used for specimen selection rather than an absolute gold standard.

Evidence from Indonesia reinforces the superior sensitivity of molecular testing relative to smear microscopy. Karuniawati et al. (2023) evaluated Xpert MTB/RIF and AFB smear microscopy against *M. tuberculosis* culture in seven Indonesian hospitals. They reported a sensitivity of 97.4% for Xpert MTB/RIF compared with 86.2% for AFB smear microscopy, demonstrating that molecular testing detected a greater proportion of culture-confirmed pulmonary TB cases.

These findings are also consistent with Roswidianah et al. (2025), who reported differences in diagnostic performance between Ziehl–Neelsen microscopy and molecular rapid testing. Datta et al. (2024) similarly emphasized that conventional staining methods remain useful in resource-constrained settings but have important sensitivity limitations, particularly when the bacillary load is low.

Accordingly, the complete negative agreement observed in this study should not be interpreted as evidence that MRT and Ziehl–Neelsen microscopy have equivalent diagnostic performance. Rather, it indicates that centrifugation with 4% NaOH did not produce additional smear-positive findings within a population selected specifically because MRT had already yielded negative results.

A more appropriate design for evaluating the diagnostic effect of centrifugation would include both MRT-positive and MRT-negative specimens and, where feasible, an independent microbiological reference method such as culture. Including specimens with different semiquantitative molecular bacillary loads would also allow investigators to determine whether concentration is more effective among specimens containing low but detectable levels of *M. tuberculosis*. Such a design would permit meaningful estimation of sensitivity, specificity, predictive values, and agreement between methods.

Overall, the present findings demonstrate that centrifugation with 4% NaOH did not change AFB detection by Ziehl–Neelsen microscopy among 50 sputum specimens with negative MRT results. The procedure appeared to reduce background mucus and debris, potentially improving microscopic readability, but this observation was not supported by quantitative smear-quality measurements. Therefore, the principal

potential benefit observed in this study relates to specimen preparation rather than enhanced diagnostic sensitivity.

Future studies should include both positive and negative specimens, larger sample sizes, standardized centrifugation protocols expressed in relative centrifugal force (×g), controlled NaOH exposure times, quantitative smear-quality scoring, blinded assessment by multiple microscopists, and an appropriate microbiological reference standard. Evaluation of cost, processing time, laboratory workload, and biosafety would further clarify whether centrifugation with 4% NaOH offers sufficient practical benefit for routine laboratory implementation.

CONCLUSION

Centrifugation using a 4% NaOH solution did not alter acid-fast bacilli (AFB) detection by the Ziehl–Neelsen method in 50 sputum specimens with negative Molecular Rapid Test (MRT) results, as all specimens remained AFB-negative before and after treatment. Although the centrifuged smears showed a cleaner microscopic background and less debris, these improvements were based on descriptive observation only and were not supported by quantitative smear-quality assessment. Therefore, the findings do not indicate an improvement in AFB detection, but suggest that centrifugation with 4% NaOH may facilitate microscopic examination by improving smear clarity. Further studies using larger and more diverse specimen groups, including MRT-positive samples, as well as standardized quantitative assessment of smear quality, are needed to confirm this potential benefit.

RECOMMENDATION

Future studies should include sputum specimens with both positive and negative Molecular Rapid Test results, involve larger sample sizes, and apply quantitative smear-quality assessment using more than one independent examiner. Comparative studies evaluating different decontamination methods and varying NaOH concentrations are also recommended to provide stronger evidence regarding the potential benefits of centrifugation for microscopic examination of tuberculosis specimens.

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