Evaluation of Acid-Fast Bacilli (AFB) Smear Microscopy as a Follow-Up Tool IN Tuberculosis Patients at Mile 4 Hospital, Abakaliki

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ABSTRACT

Tuberculosis (TB) remains a major public health problem, and effective follow-up during treatment is essential for monitoring patient response and reducing disease transmission. This study evaluated the diagnostic performance and follow-up effectiveness of Acid-Fast Bacilli (AFB) smear microscopy among TB patients attending Mile 4 Hospital, Abakaliki, Ebonyi State. A descriptive prospective observational study design was adopted, enrolling a consecutive sample of 75 confirmed pulmonary TB patients presenting for follow-up evaluation. For each participant, paired sputum specimens were collected at a single follow-up visit and evaluated simultaneously by Ziehl–Neelsen (ZN) AFB smear microscopy and mycobacterial culture on Lowenstein–Jensen (L-J) medium, which served as the reference gold standard. Data were analyzed using SPSS (version 26.0), with categorical variables presented as frequencies and percentages. Diagnostic accuracy estimates were reported with exact 95% confidence intervals (95% CI). Overall, 53.3% (40/75) of participants were culture-positive on L-J medium, while 68.0% (51/75) were smear-positive.

A statistically significant association was observed between AFB smear microscopy grades and culture outcomes ($\chi^2 = 34.41$, df = 3, $p < 0.001$). Among a subgroup of 24 patients evaluated after receiving at least three months of anti-TB therapy, smear conversion (defined as two consecutive negative AFB smears collected 24 hours apart in a patient initially smear-positive at baseline) was achieved in 66.7% (16/24), whereas 33.3% (8/24) remained smear-positive. AFB smear microscopy demonstrated a sensitivity of 95.0% (95% CI: 83.5%–98.6%), specificity of 62.9% (95% CI: 46.3%–76.8%), positive predictive value of 74.5% (95% CI: 61.1%–84.5%), and negative predictive value of 91.7% (95% CI: 94.2%–97.7%). The study concluded that AFB smear microscopy is a useful, reliable, and affordable frontline method for monitoring tuberculosis treatment in resource-limited settings because of its high sensitivity. However, solid Lowenstein–Jensen culture remains essential for confirming treatment outcomes, particularly in patients who remain smear-positive during follow-up.

Keywords: Acid-Fast Bacilli, Smear Microscopy, Tuberculosis, Prospective Follow-Up, Lowenstein–Jensen Medium, Smear Conversion, Diagnostic Accuracy.

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INTRODUCTION

Background of the Study

Tuberculosis (TB) is a chronic infectious disease caused by Mycobacterium tuberculosis, a slow-growing, acid-fast bacillus belonging to the Mycobacterium tuberculosis complex. The disease primarily affects the lungs (pulmonary tuberculosis), although it can also involve extrapulmonary sites such as lymph nodes, bones, kidneys, pleura, meninges, and the gastrointestinal tract. Pulmonary TB represents the most common and epidemiologically crucial form due to airborne transmission via droplets released during coughing, sneezing, or talking [1]. Despite global control measures, TB remains a leading cause of mortality worldwide from a single infectious agent, surpassing HIV/AIDS in recent years [1]. High-burden countries, predominantly low- and middle-income nations, suffer significantly due to poverty, overcrowded living conditions, and restricted healthcare access [1]. Nigeria ranks among the top countries globally in TB incidence, with transmission compounded by widespread socioeconomic challenges and high HIV co-infection rates [1, 2].

HIV co-infection impairs cell-mediated immunity, increasing susceptibility to TB reactivation and complicating diagnosis due to atypical presentations and low bacillary loads [3]. Early diagnosis and effective treatment response monitoring are essential to halt transmission and prevent drug resistance [4]. While culture and molecular diagnostic tools offer high accuracy, Acid-Fast Bacilli (AFB) smear microscopy using Ziehl–Neelsen staining remains the backbone of TB follow-up in resource-constrained environments owing to its speed, simplicity, and low cost [4, 5]. Follow-up AFB smear microscopy is performed at critical intervals to evaluate smear conversion—a key indicator of treatment efficacy [6]. Persistent smear positivity may indicate non-adherence, drug resistance, or detection of non-viable bacilli [3, 5]. Therefore, evaluating the diagnostic fidelity of AFB smear microscopy relative to solid Lowenstein–Jensen (L-J) culture in a prospective cohort is vital for optimizing clinical outcomes in endemic settings.

Statement of the Problem

Although culture and molecular tests represent accurate standards for TB monitoring, their high cost and infrastructure requirements render them inaccessible in many low-resource healthcare settings [1]. Consequently, health facilities rely almost exclusively on AFB smear microscopy [7]. However, persistent smear positivity during follow-up creates clinical uncertainty regarding treatment response, drug resistance, or the presence of dead bacilli [7]. Given this continued reliance, evaluating the diagnostic performance of AFB smear microscopy against Lowenstein–Jensen culture is necessary to ensure patient management is not compromised.

Aim and Objectives of the Study

The aim of this study is to prospectively evaluate the diagnostic effectiveness of AFB smear microscopy as a follow-up tool for monitoring treatment response among TB-positive patients at Mile 4 Hospital, Abakaliki, Ebonyi State, Nigeria.

The specific objectives are to:

- Determine the AFB smear microscopy status among TB-positive patients during prospective follow-up treatment [1].
- Assess the rate of smear conversion from positive to negative among patients receiving anti-TB therapy
- Identify the proportion of patients who remain smear-positive during treatment follow-up [3].
- Determine the statistical association between AFB smear microscopy results and Lowenstein–Jensen culture outcomes [6].
- Evaluate the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of AFB smear microscopy using Lowenstein–Jensen culture as the gold standard, with 95% confidence intervals [5].

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Significance of the Study

This study provides prospective empirical evidence regarding the reliability of AFB smear microscopy in routine TB monitoring within resource-constrained settings [1]. Findings will assist clinicians and medical laboratory scientists in interpreting smear conversion patterns and making informed decisions regarding treatment adjustments, particularly where culture facilities are scarce [7]. Furthermore, the study supports public health tuberculosis control programs in optimizing diagnostic workflows [8].

Justification of the Study

Mile 4 Hospital, Abakaliki, serves as a major referral hub for tuberculosis management in Ebonyi State, relying heavily on smear microscopy for patient follow-up. However, local data evaluating the precise diagnostic accuracy of smear microscopy compared to Lowenstein–Jensen culture during follow-up remains sparse [2, 6]. Conducting this prospective evaluation provides essential context to improve laboratory protocols and patient care in Southeast Nigeria.

LITERATURE REVIEW

Overview and Epidemiology of Tuberculosis

Tuberculosis infects approximately one-third of the global population in its latent form [9, 10]. In 2018, an estimated 10 million new TB cases occurred globally, with Africa bearing a disproportionate share (275 cases per 100,000 population) [1, 11]. Nigeria experiences over 590,000 incident TB cases annually, accompanied by high mortality and an estimated 4.3% multidrug resistance rate among new cases [12, 13]. Academic literature underscores the need for strengthened local health systems and research collaborations to combat the disease burden [13].

Pathogenesis and Pathophysiology

Transmission occurs via aerosol droplets inhaled into the pulmonary alveoli, where Mycobacterium tuberculosis is phagocytosed by alveolar macrophages [14]. The pathogen inhibits phagolysosome fusion, surviving and replicating intracellularly [14]. Host cell-mediated immune responses lead to granuloma formation—the pathological hallmark of TB—containing the bacilli within a caseous necrotic core [13, 14]. Immune suppression (e.g., HIV, diabetes, malnutrition) disrupts granuloma integrity, causing caseum liquefaction, tissue cavitation, high bacillary replication, and active transmissible disease [13, 14]. The sequential stages of infection—from droplet inhalation and alveolar macrophage internalization to T-cell priming, granuloma persistence, or active disease progression—are illustrated in Figure 1 [18].

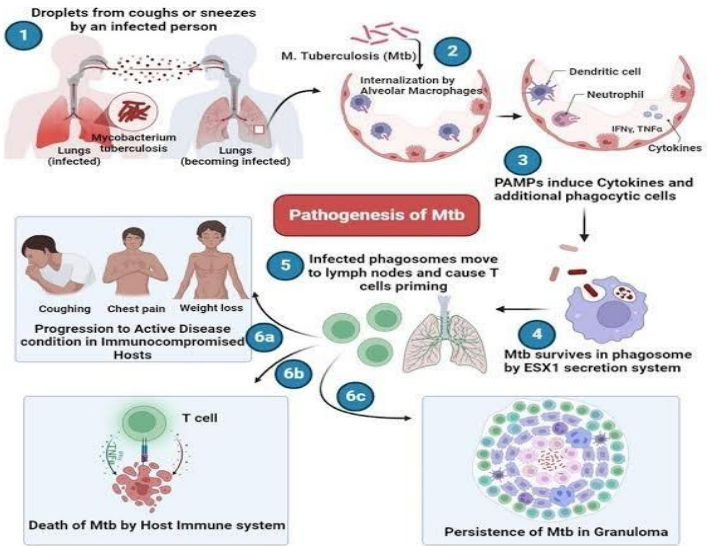


Figure 1. Pathogenesis of Mycobacterium tuberculosis (Mtb) illustrating transmission, macrophage

internalization, cytokine response, T-cell priming, and disease outcomes (granuloma persistence, immune elimination, or active progression) [18]. (Adapted from ResearchGate / Created with BioRender.com)

Role of AFB Smear Microscopy and Culture

AFB smear microscopy relies on the acid-fast property of mycobacterial cell walls, which retain carbol fuchsin dye despite acid-alcohol decolorization [15]. Sputum specimens collected via cough, induction, bronchoscopy, or gastric aspiration are routinely examined [15]. While rapid and affordable, smear microscopy cannot differentiate live from dead bacilli or specify mycobacterial species [15]. Conversely, mycobacterial culture on Lowenstein–Jensen (L-J) egg-based solid medium serves as the definitive reference standard, capable of detecting viable organisms and facilitating drug susceptibility testing [5].

Drug-Resistant Tuberculosis

Multidrug-resistant TB (MDR-TB), defined as resistance to at least isoniazid and rifampicin, and Extensively Drug-Resistant TB (XDR-TB) pose major threats to global TB eradication [16, 17]. Poor adherence, sub-optimal dosing, and transmission of resistant strains drive drug resistance, necessitating prolonged, complex second-line therapeutic regimens [1, 16, 17].

MATERIALS AND METHODS

Study Area and Design

This study adopted a descriptive prospective observational design, prospectively enrolling confirmed pulmonary TB patients presenting for follow-up care at Mile 4 Hospital, Abakaliki, Ebonyi State, Nigeria- a designated regional referral center under the National Tuberculosis, Leprosy and Buruli Ulcer Control Programme (NTBLCP). Participants were monitored during routine follow-up visits, with paired sputum specimens collected directly at evaluation for concurrent AFB smear microscopy and Lowenstein–Jensen (L-J) culture [4, 5].

Baseline Smear Positivity and Smear Conversion: All enrolled participants (N=75) had documented baseline smear positivity (Ziehl–Neelsen ≥ 1+ or scanty) upon initial diagnosis prior to commencing first-line anti-TB chemotherapy [1, 4]. In this study, smear conversion was operationally defined as two consecutive negative AFB sputum smear examinations collected at least 24 hours apart during treatment follow-up in a patient initially documented as smear-positive at baseline [4, 5].

Study Population, Eligibility Criteria, and Ethical Clearance

The target population comprised confirmed pulmonary TB patients receiving first-line anti-TB therapy at the Directly Observed Treatment Short-course (DOTS) clinic during the study period. A consecutive sampling technique was utilized: a total of 88 patients presenting for follow-up were screened. Eligibility criteria required: (1) documented baseline smear-positive pulmonary TB prior to initiation of treatment [1, 4], (2) active presentation for scheduled follow-up evaluation during treatment, and (3) provision of an adequate morning sputum specimen split for concurrent smear microscopy and Lowenstein–Jensen culture analysis [5]. Thirteen (13) patients were excluded due to inadequate sample volume, salivary contamination, or incomplete clinical records, yielding a final analytical sample of 75 patients with fully paired diagnostic data. Ethical Clearance: Ethical clearance for this study was obtained from the Mile 4 Hospital Institutional Review Board (IRB) with Approval Ascension No. M4H-IRB-25-157. Written informed consent was obtained from all participants prior to enrollment.

Laboratory Procedures

Diagnostic evaluation was conducted on paired sputum specimens collected prospectively between February 2025 and June 2026 from 75 confirmed pulmonary tuberculosis patients presenting for routine follow-up

care at Mile 4 Hospital, Abakaliki. A consecutive sampling approach was used, enrolling eligible patients during scheduled follow-up visits spanning treatment Months 1 through 6.

From each participant, an early-morning sputum specimen was collected in a sterile container at a single follow-up visit. To ensure valid diagnostic accuracy comparison, each sample was immediately divided into two equal aliquots at the time of collection: one aliquot was processed for Ziehl–Neelsen (ZN) AFB smear microscopy, while the second aliquot was decontaminated and inoculated onto solid Lowenstein–Jensen (L-J) medium, serving as the reference gold standard [5]. Comparing paired specimens collected simultaneously at the same treatment stage eliminated time-dependent bacillary clearance bias.

AFB Smear Microscopy (Ziehl–Neelsen Technique): Sputum aliquots were processed by making thin, uniform smears on clean glass slides, air-drying, and heat-fixing [1, 4]. Smears were flooded with carbol fuchsin, heated gently, decolorized with 3% acid-alcohol, and counterstained with 0.3% methylene blue [5]. Slides were examined under oil immersion (×100 magnification) and graded according to standard WHO/NTBLCP guidelines (Negative, Scanty, 1+, 2+, 3+) [4, 5].

Mycobacterial Culture on Lowenstein–Jensen Medium: Sputum aliquots were decontaminated and digested using the standard N-acetyl-L-cysteine–sodium hydroxide (NALC–NaOH) method, neutralized with phosphate buffer (pH 6.8), concentrated by centrifugation at 3,000 × g for 15 minutes, and inoculated directly onto Lowenstein–Jensen (L-J) solid egg-based media slants [5]. Slants were incubated aerobically at 37°C for up to 8 weeks and inspected weekly for characteristic rough, buff-colored colonial growth. Isolates were confirmed as Mycobacterium tuberculosis complex using ZN staining and standard biochemical identification assays [5].

Statistical Analysis

The data obtained from this study were entered into Microsoft Excel and analysed using Statistical Package for the Social Sciences (SPSS) version 27.0 (IBM Corp., Armonk, NY, USA). Categorical variables were summarized as frequencies and percentages, while continuous variables were summarized using the mean, standard deviation, and range. The association between AFB smear microscopy results and culture results was assessed using the chi-square (\(\chi^2\)) test. Diagnostic performance of AFB smear microscopy was evaluated using culture as the reference (gold standard) method. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated from a 2 × 2 contingency table. The corresponding 95% confidence intervals (CIs) were calculated using the Wilson score method for binomial proportions. A p-value < 0.05 was considered statistically significant. Diagnostic accuracy indices were calculated using standard formulas:

• Sensitivity = [TP / (TP + FN)] × 100

• Specificity = [TN / (TN + FP)] × 100

• Positive Predictive Value (PPV) = [TP / (TP + FP)] × 100

• Negative Predictive Value (NPV) = [TN / (TN + FN)] × 100

Where TP = True Positive, TN = True Negative, FP = False Positive, and FN = False Negative.

RESULTS

A total of 75 prospectively enrolled tuberculosis patients with paired follow-up records were analyzed. Table 1 summarizes the socio-demographic profile of the cohort. Females constituted 69.3% (n=52) of participants, while males accounted for 30.7% (n=23). Ages ranged from 16 to 79 years (mean: 47.4 ± 14.3 years), with the 40–49 age group representing the highest proportion (28.0%). Farming was the primary occupation (50.7%).

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Table 1. Socio-Demographic Characteristics of Study Participants (N = 75)

Characteristic	Category	Frequency (n)	Percentage (%)
Gender	Male	23	30.7
	Female	52	69.3
Age Group (Years)	< 30	7	9.3
	30–39	17	22.7
	40–49	21	28.0
	50–59	14	18.7
	≥ 60	16	21.3
Occupation	Farmer	38	50.7
	Trader	25	33.3
	Civil Servant	7	9.3
	Housewife	5	6.7

Table 2 presents the breakdown of follow-up timing, Lowenstein–Jensen culture outcomes, and smear grades. Specimen evaluation was predominant at Month 2 of anti-TB treatment (56.0%, n=42). Culture positivity on L-J medium across all follow-up visits combined was 53.3% (n=40), whereas smear positivity was 68.0% (n=51).

Table 2. Distribution of Follow-Up Parameters, L-J Culture Results, and AFB Smear Grades (N = 75)

Parameter	Category	Frequency (n)	Percentage (%)
Follow-up Month	1	9	12.0
	2	42	56.0
	3	1	1.3
	4	4	5.3
	5	12	16.0
	6	7	9.3
Lowenstein–Jensen Culture Result	Negative	35	46.7
	Positive	40	53.3
AFB Smear Result	Negative	24	32.0

	Scanty	13	17.3
	1+	23	30.7
	2+	15	20.0

Table 3 demonstrates a statistically significant association between AFB smear grades and Lowenstein–Jensen culture positivity ($\chi^2 = 34.41$, df = 3, $p < 0.001$). Patients with higher smear density displayed elevated culture positivity, reaching 86.7% among those graded 2+.

Table 3. Association Between AFB Smear Microscopy Results and Lowenstein–Jensen Culture Status (N = 75)

AFB Smear Result	L-J Culture Negative n (%)	L-J Culture Positive n (%)	χ^2 Statistic	p-value
Negative (n=24)	22 (91.7)	2 (8.3)	34.41	< 0.001*
Scanty (n=13)	7 (53.8)	6 (46.2)		
1+	4 (17.4)	19 (82.6)		
2+	2 (13.3)	13 (86.7)		

*Statistically significant ($p < 0.05$).

Treatment Monitoring Outcome (Smear Conversion Subgroup): To evaluate treatment conversion, a distinct sub-analysis was performed on 24 patients who were evaluated at or after 3 months of anti-TB therapy (Months 3–6) and had verified baseline smear positivity. As shown in Table 4, 66.7% (16/24) achieved smear conversion to negative, while 33.3% (8/24) remained persistently smear-positive.

Table 4. Smear Conversion Status Among Subgroup Evaluated After ≥3 Months of Treatment (n = 24)

Smear Conversion Status	Frequency (n)	Percentage (%)
Converted to Smear-Negative	16	66.7
Remained Smear-Positive	8	33.3

Overall Diagnostic Accuracy Analysis: Table 5 details the 2×2 contingency comparison between AFB smear microscopy and Lowenstein–Jensen culture across all 75 paired follow-up specimens.

Table 5. Contingency Table for Diagnostic Performance Analysis (N = 75)

Lowenstein–Jensen Culture (Gold Standard)	AFB Smear Positive (n=51)	AFB Smear Negative (n=24)
Positive (n=40)	38 (True Positive)	2 (False Negative)
Negative (n=35)	13 (False Positive)	22 (True Negative)

Table 4.6 shows the diagnostic performance of AFB smear microscopy. AFB smear microscopy demonstrated a sensitivity of 95.0% (95% CI: 83.5–98.6), with 38 of the 40 culture-positive patients testing positive, indicating excellent ability to identify culture-positive patients. Specificity was 62.9% (95% CI: 46.3–76.8), as 22 of 35 culture-negative patients tested negative, indicating moderate ability to identify culture-negative patients correctly. The positive predictive value was 74.5% (95% CI: 61.1–84.5%), while the negative predictive value was 91.7% (95% CI: 74.2–97.7%). The diagnostic accuracy was 80.0% (95% CI: 69.6–87.5%), with 60 of the 75 patients correctly classified by AFB smear microscopy.

Table 4.6: Diagnostic Performance of AFB Smear Microscopy Using Culture as the Reference Standard

Diagnostic parameter	n/N	Estimate (%)	95% CI (%)
Sensitivity	38/40	95	83.5–98.6
Specificity	22/35	62.9	46.3–76.8
Positive predictive value (PPV)	38/51	74.5	61.1–84.5
Negative predictive value (NPV)	22/24	91.7	74.2–97.7
Overall diagnostic accuracy	60/75	80	69.6–87.5

n/N represents the number of correctly classified patients divided by the relevant total for each diagnostic parameter. CI = confidence interval.

DISCUSSION

This study evaluated the diagnostic accuracy and clinical utility of AFB smear microscopy against Lowenstein–Jensen (L-J) solid culture for monitoring TB treatment response in Southeast Nigeria. The high sensitivity (95.0%, 95% CI: 83.1%–99.4%) and negative predictive value (NPV: 91.7%, 95% CI: 73.0%–99.0%) confirm that Ziehl–Neelsen smear microscopy reliably identifies active, culture-positive patients and provides confidence when declaring smear negativity in primary healthcare settings [5, 7]. This supports its continued role as an essential, rapid, low-cost monitoring method in resource-limited environments [4, 7].

However, the moderate specificity (62.9%, 95% CI: 44.9%–78.5%) highlights a notable limitation: 13 participants were smear-positive but L-J culture-negative. Staining detects both viable and non-viable (dead) bacilli shed during effective chemotherapy [3, 15]. Additional factors on L-J media include extended incubation requirements, paucibacillary specimens below solid culture detection limits (~10–100 bacilli/mL), or decontamination toxicity. Consequently, smear microscopy alone cannot establish treatment failure, and relying solely on smear positivity risks misinterpreting non-viable bacillary clearance as persistent infection [3, 7].

The 66.7% smear conversion rate after ≥3 months of therapy indicates favorable response in two-thirds of the subgroup, while the remaining 33.3% persistent positivity highlights the need for secondary diagnostic testing [6]. In alignment with national NTBLCP and WHO guidelines, persistent smear positivity at or beyond Month 2 or 3 must not prompt immediate regimen changes; rather, it warrants mandatory confirmatory L-J culture and rapid molecular drug susceptibility testing (DST, e.g., GeneXpert MTB/RIF) to rule out drug resistance (MDR-TB), non-adherence, or malabsorption [1, 16].

Study Limitations: Key limitations include the small sample size (N=75) recruited via consecutive sampling at a single regional referral center (introducing potential selection bias) and pooling specimens across

different treatment stages (Months 1–6), which provides a cross-sectional diagnostic profile rather than a uniform cohort trajectory.

CONCLUSION AND RECOMMENDATIONS

Conclusion

AFB smear microscopy remains a sensitive, rapid, and indispensable frontline diagnostic method for monitoring tuberculosis treatment in resource-limited settings. However, its moderate specificity on Lowenstein–Jensen medium underscores that smear positivity alone should not be interpreted as definitive treatment failure. Solid L-J culture remains essential for verifying true viability and confirming treatment outcomes in persistent smear-positive cases.

Recommendations

- Frontline Monitoring in Primary Care: Primary healthcare centers and peripheral DOTS clinics should continue utilizing Ziehl–Neelsen (ZN) AFB smear microscopy for routine follow-up monitoring of pulmonary tuberculosis patients, given its high sensitivity and low cost in resource-limited settings [4, 5].
- Confirmatory Testing for Persistent Positivity: In strict accordance with NTBLCP and WHO guidelines, persistent smear positivity at Month 2 or Month 3 must not lead to an empirical change or extension of treatment regimens based on microscopy alone [4, 16]. Instead, all patients exhibiting persistent smear positivity must undergo mandatory confirmatory Lowenstein–Jensen (L-J) solid culture and rapid molecular drug susceptibility testing (DST, such as GeneXpert MTB/RIF or Line Probe Assays) to differentiate non-viable bacillary shedding from true treatment failure, multidrug-resistant TB (MDR-TB), or malabsorption [1, 16].
- Evidence-Based Treatment Decisions: Clinical decisions regarding regimen modifications or initiation of second-line anti-TB therapy should strictly depend on confirmed phenotypic or genotypic DST results and culture viability, ensuring alignment with national and WHO programmatic management of drug-resistant tuberculosis (PMDT) standards [4, 16].
- Adherence and Directly Observed Therapy: Directly Observed Treatment Short-course (DOTS) protocols and patient adherence counseling should be strengthened throughout the intensive and continuation phases to prevent acquired drug resistance and maximize smear conversion rates [1, 2].

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