Eliya et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 277 - 285 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2489. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Impact of sputum transit time on TB isolation and culture contamination rate

*[1,2]Eliya, T. T., [1,2]Ajide, B. A., [1]Ugwu, C., [1]Emmana, B., [1]Ishaku, P., [1]James, E., and [1,3]John, B.

[1]Zankli Research Centre, Bingham University, Karu, Nasarawa State, Nigeria
[2]Biological Sciences Department, Bingham University, Karu, Nasarawa State, Nigeria
[3]Community Medicine and Primary Health Care Department, Bingham University, Karu, Nasarawa State, Nigeria
*Correspondence to: iliya.taryuta@binghamuni.edu.ng; Tel: +2347034229746

Abstract:

Background: Tuberculosis (TB) culture is the 'gold standard' test for detecting *Mycobacterium tuberculosis* and enables comprehensive drug susceptibility testing. However, culture contamination remains a challenge in TB culture, resulting in significant loss of time and resources, and potentially leading to poor treatment outcomes due to incorrect clinical decision-making. The objective of this study is to assess the effect of sputum transit time on contamination rate and the yield of *M. tuberculosis.*
Methodology: We conducted a prospective analysis of all samples received for TB culture at a Reference Laboratory from January to December 2021. Samples were decontaminated by the standard N-Acetyl L-cysteine (NALC)/Sodium Hydroxide (NaOH) method and inoculated on Lowenstein-Jensen (LJ) solid media. The number of days from sample collection to processing was documented for all 988 valid samples received within the study period. A univariable analysis was used to explore the relationship between transit time and contamination rate. Percentages and Odds ratios with associated 95% confidence intervals were reported.
Results: Of 988 diagnoses and follow-up samples received, 508 (58.7%) were from males, while 14 (1.4%) were from children ≤ 14 years of age. Overall, 56 (5.7%) samples were contaminated, of which 17 (30.4%) were positive for AFB. A contamination rate of 4.1%, 7.1%, and 8.3% was recorded for Optimal (0-7 days), Delayed (8-14 days), and Extended delay (>15 days) samples, respectively. Compared to samples analyzed within optimal transit time, those with Delayed and Extended delay transit times had greater odds of being contaminated, with OR of 1.811 (95% CI=0.997-3.290) and 2.148 (95% CI=0.983-4.688), respectively.
Conclusion: The study revealed that TB culture contamination rate tends to increase with the number of days taken for sample movement from collection to processing. However, the association detected fell slightly short of achieving statistical significance. It is also instructive that about one in three contaminated samples were positive for AFB, which could have far-reaching implications for the clinical decision-making and management of persons being treated for various forms of TB. Investment in innovative approaches to ensure that all samples for TB culture reach the testing laboratories within the optimal period is likely to save time, resources, and lives.

Keywords: *Mycobacterium tuberculosis*, Tuberculosis-culture, contamination-rate, transit-time.

Received Jan 20, 2026; Revised Mar 20, 2026; Accepted Mar 21, 2026

Impact du temps de transit des expectorations sur l'isolement de la tuberculose et le taux de contamination des cultures

*[1,2]Eliya, T. T., [1,2]Ajide, B. A., [1]Ugwu, C., [1]Emmana, B., [1]Ishaku, P., [1]James, E., et [1,3]John, B.

[1]Centre de Recherche Zankli, Université Bingham, Karu, État de Nasarawa, Nigéria
[2]Département des Sciences Biologiques, Université Bingham, Karu, État de Nasarawa, Nigéria
[3]Département de Médecine Communautaire et de Soins de Santé Primaires, Université Bingham, Karu, État de Nasarawa, Nigéria
*Correspondance : iliya.taryuta@binghamuni.edu.ng; Tél: +2347034229746

Résumé:

Contexte: La culture de la tuberculose (TB) est la méthode de référence pour la détection de Mycobacterium tuberculosis et permet de réaliser des tests complets de sensibilité aux médicaments. Cependant, la contamination des cultures reste un défi majeur pour la recherche de la tuberculose, entraînant des pertes importantes de temps et de ressources, et pouvant potentiellement compromettre l'efficacité du traitement en raison d'erreurs de jugement clinique. L'objectif de cette étude est d'évaluer l'influence du temps de transit des expectorations sur le taux de contamination et le rendement en mycobactéries tuberculeuses (M. tuberculosis).
Méthodologie: Nous avons mené une analyse prospective de tous les échantillons reçus pour la recherche de la tuberculose dans un laboratoire de référence, de janvier à décembre 2021. Les échantillons ont été décontaminés par la méthode standard à la N-acétyl-L-cystéine (NALC)/hydroxyde de sodium (NaOH) et ensemencés sur milieu solide de Löwenstein-Jensen (LJ). Le délai entre le prélèvement et le traitement des échantillons a été enregistré pour les 988 échantillons valides reçus pendant la période d'étude. Une analyse univariée a été utilisée pour explorer la relation entre le temps de transit et le taux de contamination. Les pourcentages et les rapports de cotes avec leurs intervalles de confiance à 95% ont été rapportés.
Résultats: Sur 988 diagnostics et échantillons de suivi reçus, 508 (58,7%) provenaient de garçons et 14 (1,4%) d'enfants de 14 ans ou moins. Au total, 56 échantillons (5,7%) étaient contaminés, dont 17 (30,4%) étaient positifs pour les BAAR. Les taux de contamination étaient respectivement de 4,1%, 7,1% et 8,3% pour les échantillons ayant bénéficié d'un délai de transit optimal (0 à 7 jours), retardé (8 à 14 jours) et prolongé (> 15 jours). Comparativement aux échantillons analysés dans les délais de transit optimaux, ceux ayant bénéficié d'un délai de transit retardé ou prolongé présentaient un risque de contamination plus élevé, avec un OR de 1,811 (IC à 95%: 0,997-3,290) et de 2,148 (IC à 95%: 0,983-4,688), respectivement.
Conclusion: L'étude a révélé que le taux de contamination des cultures de tuberculose tend à augmenter avec le nombre de jours nécessaires pour acheminer l'échantillon, du prélèvement à l'analyse. Cependant, l'association observée n'a pas tout à fait atteint le seuil de signification statistique. Il est également important de noter qu'environ un tiers des échantillons contaminés étaient positifs pour les BAAR, ce qui pourrait avoir des conséquences majeures sur la prise de décision clinique et la prise en charge des personnes traitées pour différentes formes de tuberculose. Investir dans des approches innovantes pour garantir que tous les échantillons destinés à la culture de tuberculose parviennent aux laboratoires d'analyse dans les délais optimaux permettra probablement de gagner du temps, d'économiser des ressources et de sauver des vies.

Mots-clés: *Mycobacterium tuberculosis*, culture de tuberculose, taux de contamination, délai de transit.

Introduction:

Tuberculosis (TB) continues to pose a formidable challenge to global public health, particularly in developing countries where healthcare infrastructure and resources remain limited (1). Despite significant strides in diagnosis and treatment, the disease persists with alarming prevalence (1,2). It is estimated that nearly one-third of the world's population harbors latent infection with *Mycobacterium tuberculosis* (MTB), the causative agent of TB (3). Each year, between 8 and 9 million new cases are reported, and approximately 3 million people succumb to the disease (4). These statistics underscore the urgent need for reliable diagnostic methods that can support early detection and effective treatment.

While molecular diagnostic tools have revolutionized TB detection by significantly reducing the time to diagnosis, culture remains the gold standard for confirming infection and conducting comprehensive drug susceptibility testing (DST) (5). Culture methods not only enhance diagnostic yield but also provide critical insights into the viability and resistance profile of MTB strains (6). Among the various culture techniques, the BACTEC MGIT 960 system has demonstrated superior performance, isolating mycobacteria 7–10 days faster than traditional methods such as Lowenstein-Jensen (LJ) and Middlebrook 7H10 culture media (7). In terms of both isolation rate and number of isolates, BACTEC MGIT 960 has proven to be more efficient and reliable (7).

However, the success of TB culture is influenced by several pre-analytical factors, one of the most critical being the transit time of sputum specimens from collection to processing. In remote or resource-limited settings, delays in transportation and lack of refrigeration can compromise specimen integrity, leading to increased contamination rates and reduced culture positivity (8). Interestingly, studies (9,10) have shown that sputum samples stored at −20°C for extended periods can still yield high recovery rates of MTB, even without the use of preservatives. This finding presents a cost-effective alternative for sputum storage in epidemiological and drug resistance studies, especially in low-resource environments.

A study by David et al., (11) reported a median transit time of 18 days between specimen collection and processing. Remarkably, MTB was successfully isolated from 94.4% of the samples, with only 3.7% failing to grow any mycobacteria (12). Even specimens that experienced breakthrough contamination were salvaged through a second decontamination procedure. These results suggest that single morning sputum specimens, even when refrigerated for extended periods without preservatives and transported without

refrigeration, can maintain diagnostic integrity.

Innovative transportation methods such as drone delivery, have also been explored to address logistical challenges in specimen transport. One study evaluating the impact of drone transportation on sputum quality found acceptable agreement between drone-transported samples and controls, indicating that drone delivery did not compromise specimen quality (13). The authors advocate for further research to validate this method over longer durations, potentially paving the way for its broader adoption in TB control programs.

The choice of sputum preservation method also plays a crucial role in culture outcomes. OMNIgene-Sputum (OM-S) treatment has been shown to reduce contamination rates compared to standard decontamination procedures (SDP) (14). In same-day and 5-day transit arms, contamination rates were 2% and 12% for OM-S-treated cultures, respectively, compared to 15% and 27% for SDP-treated cultures. Moreover, MTB recovery in OM-S-treated LJ cultures was significantly higher, and more in same-day and 5-day arms, respectively, than in SDP-treated cultures (15,16,17). However, OM-S-treated samples showed lower recovery rates in MGIT cultures and a 1-log reduction in viable MTB count after five days, suggesting that while OM-S improves culture quality, extended transit times may still affect bacterial viability (16,18).

Culture positivity tends to decline with increased transit time, with week 2 and week 3 specimens showing higher contamination and negativity rates compared to day-one samples (19). This trend likely reflects the diminishing viability of MTB and the proliferation of contaminating organisms over time. In a study involving 816 sputum samples preserved in cetylpyridinium chloride (CPC), 84.7% yielded MTB, while 11.9% showed no growth, 2.6% were contaminated, and 0.8% grew non-tuberculous mycobacteria (20). Notably, samples processed within two weeks showed 88.6% culture positivity, whereas those processed beyond two weeks experienced a significant decline.

OM-S has also demonstrated the ability to maintain MTB viability for up to three weeks without refrigeration, resulting in contamination rates below 0.5% (21). However, samples stored for more than two weeks exhibited delayed culture positivity, indicating that prolonged storage, even with preservatives, can affect diagnostic timelines. For sputum specimens requiring transit times of up to seven or eight days, the CPC-NaCl method has shown advantages over the standard NaOH method, reducing contamination and enhancing culture positivity on solid media within 1–2 weeks of collection (22).

Given these findings, this study seeks to evaluate the impact of sputum transit time on TB culture contamination rates. Specifically, it aims to estimate the average duration from specimen collection to arrival at the reference laboratory in north central Nigeria, and to assess how this transit time influences both contamination rates and the yield of *M. tuberculosis*. Understanding these dynamics is essential for optimizing specimen handling protocols and improving diagnostic outcomes, particularly in settings where timely transportation and refrigeration are not guaranteed.

Materials and method:

Study Setting:

The study was conducted in Zankli Research Center and health facilities located across the North Central region of Nigeria, a zone comprising both urban and rural communities with varying levels of access to tuberculosis diagnostic services. Participants were recruited from the period of 21st May – 30th October 2025 in selected primary, secondary, and tertiary health facilities where individuals presenting with symptoms suggestive of TB were undergoing routine screening. Rifampicin-resistant cases were then referred for TB culture and drug susceptibility testing at Zankli Research Center, as the zonal TB Reference laboratory. Sputum samples collected at these facilities were transported to the Zankli Research Centre, located within Bingham University, Karu, Nasarawa State, for TB culture and further laboratory analysis.

Zankli Research Centre is a specialized diagnostic and research laboratory equipped with biosafety level 2+ (BSL-2+) facilities, and it serves as a referral laboratory for TB diagnostics, including culture, drug susceptibility testing, and molecular assays. The centralization of TB culture testing at Zankli Research Centre ensured the application of standardized laboratory procedures, minimized inter-laboratory variability, and enabled accurate determination of culture outcomes. This setting allowed for the assessment of sputum transit times from peripheral health facilities to the central laboratory, which was a key variable in the study.

Ethical considerations:

Ethical approval was obtained (NHREC/21/05/2005/01501) from the Bingham University Institutional Review Board (IRB) before commencement. Eligible participants were informed about the study objectives, procedures, potential benefits, and risks in a language they understood. Written informed consent was obtained from all participants before enrolment. For participants under 18 years of age, assent was obtained along

with parental or guardian consent.

All sputum samples were collected as part of routine TB diagnostic services, with no additional invasive procedures performed for research purposes. Demographic and clinical data were obtained using a structured data collection form, and each participant was assigned a unique identification code to ensure anonymity. No personal identifiers (e.g., names, addresses, or phone numbers) appeared in any database or publication. The study adhered to ethical principles. All data were stored securely in password-protected electronic databases and locked filing cabinets for physical forms, accessible only to authorized study personnel. Participants were informed of their right to withdraw from the study at any time without any impact on their access to routine medical care.

Potential risks were minimal and related mainly to the collection of sputum samples, which is standard for TB diagnosis and may cause mild discomfort. The potential benefits included improved understanding of the effect of sputum transit time on culture contamination rates, which could inform national TB diagnostic guidelines, improve laboratory efficiency, and enhance patient outcomes. Results were disseminated in aggregate form only, with no possibility of linking findings to individual participants or facilities.

Study design:

This study utilized a prospective approach to determine the period in days from the collection of sputum samples, their arrival in the laboratory, and their processing, and to further assess the impact of the transportation period in days on the TB culture contamination rate and the yield of *M. tuberculosis* complex

Study population and sampling strategy:

The study population comprised individuals of all genders and age groups who presented to participating health facilities in north-central Nigeria with confirmed TB-positive results on the GeneXpert platform, as the baseline testing in Nigeria, and were referred for TB culture. The facilities included primary, secondary, and tertiary healthcare centers across urban, peri-urban, and rural areas.

Inclusion and exclusion criteria:

Inclusion criteria included individuals with confirmed TB-positive, rifampicin-resistant results on the GeneXpert platform, who could provide adequate sputum specimens for TB culture (at least 3mls), and willingness to provide written informed consent (or assent with parental/guardian consent for participants under 18 years). Exclusion criteria included inability or unwillingness to provide informed consent, unable to provide adequate sputum volume and TB positive but rifampicin susceptible cases.

Sample size and justification:

A total of 980 participants were targeted for enrolment based on the laboratory's annual TB culture testing capacity and the average monthly number of samples received from North Central facilities. This sample size was considered adequate to allow meaningful statistical analysis of the association between sputum transit time and culture contamination rates, assuming an estimated contamination prevalence of 13.5% from a previous study (23), a confidence level of 95%, and a margin of error of 5%.

Sampling strategy:

A consecutive sampling approach was used. All eligible patients presenting to the participating health facilities during the study period and meeting the inclusion criteria were invited to participate until the end of the recruitment period from 21st May to 30th October 2025.

Sample and data collection:

Biological sample collection:

The biological samples collected in this study were expectorated sputum specimens from participants bacteriologically confirmed with drug resistant TB on the GeneXpert platform, which were identified by trained health workers at each facility. Each participant was instructed in proper sputum production, following the National TB and Leprosy Control Programme (NTBLCP) Guidelines (24), to ensure collection of high-quality, mucoid or purulent specimens rather than saliva. For each participant, one sputum sample was collected at the time of the participant's enrollment.

The sample was collected in a sterile, leak-proof, screw-cap container (Falcon 50 mL conical tubes) containing no preservatives. The target volume per sample was 3–5 mL of sputum. After collection, sputum specimens were immediately labeled with a unique participant identification code and placed in a triple-packaging system compliant with WHO biosafety requirements (25). Samples were stored in a cooler box containing ice packs, maintaining a temperature between 2–8°C during temporary storage at the health facility and throughout transportation to the Zankli Research Centre, Karu, Nasarawa State. Transit time was recorded from sample collection to arrival at the laboratory.

Upon receipt at the BSL-2+ laboratory of Zankli Research Centre, samples were logged and processed promptly for TB culture. If immediate processing was not possible, they were stored at 4°C for a maximum of 48 hours before culture.

Data collection tools:

Demographic and clinical data were collected using a structured case report form (CRF) developed specifically for this study. The CRF captured the following variables: Participant demographics (age, sex, address), Clinical history (symptoms, HIV status, prior TB history), Sample collection details (date, time, facility), and laboratory results. The CRF was pretested with a small subset of participants (n=10) to assess clarity, relevance, and ease of use in the local context. Feedback was used to refine the wording and sequence of questions. For HIV status, data were cross-checked with facility HIV registers when available.

Data collection procedure:

Trained health workers conducted in-person interviews with consented participants in private spaces at the health facilities, using English or local languages (Hausa, Tiv, Idoma) depending on participant preference. Where necessary, a trained interpreter assisted to ensure accuracy and cultural sensitivity. Completed CRFs were reviewed daily by field supervisors for completeness and accuracy before sample dispatch.

Decontamination and culture procedure:

The N-acetyl L-cysteine (NALC)/sodium hydroxide (NaOH) standard method was used to process the sputum samples in the BSL-2+ lab (26). To prevent the loss of mycobacterial viability, NaOH was neutralized by adding phosphate buffer after 15 min. The mixture was centrifuged at 3000_g for 15 min, and the supernatant was discarded. The sediment was used for concentrated AFB smear and Solid culture inoculation on solid media. The Ziehl-Neelsen (ZN) acid-fast staining was carried out on the smear, and the AFB smears were graded as scanty (1-9 AFB/100 fields), 1+ (10-99/100 fields), 2+ (1-10 AFB/ field), or 3+ (more than 10 AFB/field) (27).

About 0.5 ml of processed sample was inoculated onto prepared L-J slants and incubated aerobically. Weekly observation for 8 weeks was made to check for growth. ZN staining was performed on the isolates of the positive cultures to detect the presence of acid-fast bacilli

BIO-LINE SD Ag MPT64 TB test:

A loop full of the culture colonies was emulsified in 100 µl sample buffer of BIO-LINE SD Ag MPT64 TB (SD Biosensor, Gyeonggbuk-do, Korea) in a cryovial, and the content was added to the well of the rapid kit cassette. Inoculated immunochromatographic cassettes were kept at room temperature for 15 min in a Class II Biosafety cabinet and examined for the presence of the control and test bands. The appearance of the band in the "C" region confirmed the validity of the test. The additional appearance of the band in the "T" region was interpreted as positive for the presence of MPT64 Ag antigen in the test culture. No band in the "C" region was interpreted as an invalid test. Standard reference strain *M. tuberculosis* complex was used as a positive control (28).

Data analysis:

Data were initially captured using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA) and subjected to double-entry verification to minimize transcription errors. Cleaning procedures included screening for missing or inconsistent values, verifying dates of sample collection and receipt, and ensuring correct coding of categorical variables. The cleaned dataset was exported to IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) for statistical analysis.

Descriptive statistics were used to summarize demographic, clinical, and laboratory characteristics. Categorical variables (e.g., sex, age category, HIV status, transit time category, and contamination status) were presented as frequencies and percentages. Continuous variables (e.g., transit time in days) were also analyzed. To determine the association between sputum transit time and culture contamination rate, samples were categorized into three groups: Optimal (0–7 days), Delayed (8–14 days), and Extended Delay (>15 days). Bivariate analysis was performed to estimate odds ratios (OR) with 95% confidence intervals (CI), using the Optimal transit time category as the reference group. This allowed for quantification of the relative odds of contamination for Delayed and Extended Delay samples.

Results:

Socio-demographic characteristics and HIV status of the study participants with respect to sputum culture results:

Table 1 presents the distribution of solid TB culture results, including contaminated samples, stratified by age, gender, and HIV status. Among participants aged 0–14 years, 28.6% (4/14) had culture-positive results, while 71.4% (10/14) were negative, and no contaminated samples were recorded. In contrast, participants aged 15 years and above had a lower proportion of culture positivity (16.8%, 164/974), 77.4% (754/974) were negative, and a contamination rate of 5.7% (56/974). The difference in culture positivity and contamination rate was not statistically significant with respect to age group (p= 0.3704). With respect to gender, males showed a slightly higher culture positivity rate (19.0%, 110/580) compared to females (14.2%, 58/408).

Table 1: Sociodemographic distribution of participants and HIV status with respect to solid TB culture results and contamination rate

Characteristics	Result of solid culture (%)			Total	x^2	*p* value
	Positive	Negative	Contaminated			
Age group (years)						
0-14	4 (28.6)	10 (71.4)	0	14	1.986	0.3704
> 14	164 (16.8)	754 (77.4)	56 (5.7)	974		
Gender						
Male	110 (19.0)	430 (74.1)	40 (6.9)	580	8.766	0.0125*
Female	58 (14.2)	334 (81.9)	16 (3.9)	408		
HIV status						
Positive	20 (15.7)	102 (80.3)	5 (3.9)	127	2.393	0.6639
Negative	130 (16.7)	600 (77.2)	47 (6.0)	777		
Unknown	18 (21.4)	62 (73.8)	4 (4.8)	84		

* = statistically significant at $p<0.05$

Contamination was also more frequent among males (6.9%) than females (3.9%). Culture positivity and contamination rates were significantly higher in males than females (p= 0.0125).

When stratified by HIV status, culture positivity was observed in 15.7% (20/127) of HIV-positive participants, 16.7% (130/777) of HIV-negative participants, and 21.4% (18/84) of those with unknown HIV status. Contamination rates were highest among HIV-negative participants (6.0%), followed by those with unknown status (4.8%), and lowest among HIV-positive participants (3.9%). The difference in culture positivity and contamination rates was not significantly different with respect to HIV status (p= 0.6639).

Sputum transit time in relation to solid culture positivity and culture contamination:

Table 2 shows the distribution of solid TB culture results according to the number of days between sputum sample collection and laboratory processing. Samples processed within the optimal transit time of 0–7 days had the highest proportion of culture positivity (19.3%, 100/517) and the lowest contamination rate (4.1%, 21/517). For samples processed after a delay of 8–14 days, culture positivity declined to 14.2% (50/351), while contamination rates increased to 7.1% (25/351). Similarly, samples with extended delays of more than 14 days showed a culture positivity rate of 15.0% (18 /120) and the highest contamination rate 8.3% (10/120).

Bivariate analysis, using the Optimal category as the reference, indicated that Delayed samples (8–14 days) had 1.81 times higher odds of contamination (95% CI: 0.997–3.290), while Extended delays (>14 days) were associated with 2.15 times higher odds of contamination (95% CI: 0.983–4.688). Although both categories showed a trend toward increased contamination risk with longer transit times, the associations narrowly missed conventional statistical significance (x^2=8.084, df=4, p=0.0662)

Smear microscopy results with solid culture outcomes and contamination rate:

Table 3 compares Acid-Fast Bacilli (AFB) smear microscopy results with solid TB culture outcomes, highlighting the proportion of smear-positive but culture-contaminated samples. Among AFB-negative samples, culture positivity was 7.3% (56/762), with the majority yielding negative culture results (87.5%) and a contamination rate of 5.1% (39/762).

In smear-positive categories, culture positivity increased progressively with higher smear grades. Samples graded as "scanty" on AFB microscopy had 24.2% (8/33) solid culture positivity and a 6.0% contamination rate, while those graded as 1+ showed 40.2% (33/82) culture positivity and 7.3% contamination. Samples graded 2+ demonstrated 52.0% (26/50) culture positivity and 8.0% contamination, while those with the highest smear grade (3+) yielded 75.0% (45/60) culture positivity and 8.3% contamination.

Notably, contamination was present across all smear categories, including high-grade smear positives, with 17 out of 56 contaminated samples being smear positive. The association between AFB microscopy with culture positivity and contamination rate was statistically significant (x^2=279.99, df=8, $p<0.0001$).

Table 2: Bivariate analysis of sputum transit time in relation to solid culture positivity and culture contamination

Days from sample collection to processing	Result of Solid Culture (%)			OR (95% CI)	x^2	*p* value
	Positive	Negative	Contaminated			
Optimal (0-7)	100 (19.3)	396 (76.6)	21 (4.1)	1.521 (1.089-378)	8.084	0.662
Delayed (8-14)	50 (14.2)	276 (78.6)	25 (7.1)	1.811 (0.997-3.290)		
Extended Delayed (**>**14**)**	18 (15.0)	92 (76.7)	10 (8.3)	2.148 (0.983-4.688)		
Total	**168 (17.0)**	**764 (77.3)**	**56 (5.7)**			

OR=Odd Ratio; CI=Confidence Interval; x^2 = Chi square

Table 3: Bivariate analysis of AFB smear results with solid culture positivity and contamination rate

AFB Result	Solid Culture Result (%)			x^2	*p* value
	Positive	Negative	Contaminated		
Negative	56 (7.3)	667 (87.5)	39 (5.1)	279.99	<0.0001*
Scanty	8 (24.2)	24 (69.0)	2 (6.0)		
1+	33 (40.2)	43 (52.4)	6 (7.3)		
2+	26 (52.0)	20 (40.0)	4 (8.0)		
3+	45 (75.0)	10 (16.7)	5 (8.3)		
Total	**168 (17.0)**	**764 (77.3)**	**56 (5.7)**		

x^2=Chi square 279.99, * = statistically significant at $p<0.05$

Discussion:

This study assessed the impact of sputum sample transit time on the positivity of TB culture and contamination rates among patients with drug-resistant TB in northcentral Nigeria. The key findings show that shorter transit times (0–7 days) were associated with higher culture positivity (19.3%) and lower contamination rate (4.1%), whereas delays of more than 7 days were linked to reduced positivity and increased contamination rates. Although bivariate analysis indicated increased odds of contamination with delays (OR: 1.81 for 8–14 days; OR: 2.15 for >14 days), the associations narrowly missed statistical significance (p=0.0662). Additionally, contamination was observed across all smear grades, including high-grade positives, with 17 of 56 contaminated samples being smear-positive.

Our findings reinforce existing evidence (12,13) that prolonged transit times compromise TB culture integrity. Similar studies (9, 10,13) in high-burden settings have reported that delays facilitate the overgrowth of contaminating flora, reduce MTBC viability, and diminish diagnostic yield. The trend of increasing contamination with longer delays, even without statistical significance, is operationally important, as culture loss directly affects drug susceptibility testing (DST) capacity (29). The observation that a third of contaminated samples were smear-positive echoes earlier reports (30), highlighting that even samples with high bacillary load are vulnerable to contamination if processing is delayed.

The demographic patterns showing higher culture positivity in children (28.6%) compared to adults (16.8%) may reflect sample prioritization or differences in clinical presentation. The slightly but significantly higher positivity and contamination among males may be linked to higher TB burden or occupational exposure risks in this group as reported by a study (31). In HIV-positive participants, lower positivity rates (15.7%) are consistent with the lower bacillary load seen in immunocompromised hosts, while lower contamination (3.9%) might reflect more cautious laboratory handling.

The correlation between smear grading and culture positivity observed here follows well-documented (32) trends; higher smear grades correspond with higher culture yields. However, the occurrence of contamination even in 3+ smear samples underscores that sample quality is not the only part of the equation; timely and proper handling remains crucial. These findings have operational and policy implications. Delays in sputum transport should be minimized by strengthening specimen referral networks, using dedicated transport systems, and considering innovations such as same-day courier services or molecular testing closer to the point of care. Where culture remains essential, especially for DST, the introduction of sample-preserving media could help mitigate contamination risk during extended transit.

From research perspective, further stu-

dies are needed to quantify the impact of environmental and transport conditions, as well as to evaluate cost-effective interventions to reduce delays. For policy, our findings support the integration of rapid molecular diagnostics at peripheral health facilities to reduce reliance on central laboratories for initial diagnosis.

This study had some limitations. First, as a prospective observational design, it cannot establish causality between transit time and contamination. Second, environmental factors during transport, such as temperature and storage conditions, were not systematically recorded, limiting the ability to directly link them to culture outcomes. Third, the relatively small number of pediatric samples constrained subgroup analyses. Additionally, while logistic regression trends were consistent, some associations narrowly missed statistical significance, likely due to sample size limitations in the Delayed categories.

Conclusion:

In summary, the study demonstrates that longer sputum transit times are associated with lower TB culture positivity and higher contamination rates, with potentially significant implications for TB diagnosis and treatment decision-making. Contamination in smear-positive samples represents a lost opportunity for DST and optimal patient management. Addressing transport delays through improved logistics, sample preservation methods, and expanded access to rapid diagnostics will be essential for improving TB diagnostic performance and reducing missed opportunities in care.

Acknowledgements:

The authors gratefully acknowledge the Director and all staff of Zankli Research Center for their unwavering support and for providing an enabling environment to conduct the laboratory work for this study. We extend our appreciation to the National Tuberculosis, Leprosy and Buruli Ulcer Control Programme (NTBLCP), the TB programme managers, the quality assurance officer, and the dedicated staff of the selected health facilities across North Central Nigeria for their collaboration and commitment throughout the study. We also thank the Institute of Human Virology Nigeria (IHVN) for their technical and operational support.

Contributions of authors:

TTE and CU conceptualized the study, developed the study protocol, and drafted the manuscript; AB and EJ finalized and proofread the manuscript; BE, TTE and PI performed the laboratory work; CU and TTE performed the data analysis. BJ reviewed the manuscript and provided overall guidance. All authors read and approved the manuscript submitted for publication.

Source of funding:

This study did not receive any external funding but received institutional support from Zankli Research Center, Bingham University, Karu, Nigeria**.**

Conflict of interest:

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Declaration:

ChatGPT 3.5 and Grammarly were utilized to brainstorm structure and phrasing during the early stages of manuscript drafting. All intellectual contributions and final content are the result of the work of the authors. The content was reviewed and edited by the authors, who take full responsibility for its accuracy

Data availability:

The authors will make the data supporting the findings of this study available through the corresponding author (TTE) upon reasonable request, following our data sharing policy.

References:

1. Suvvari, T. K. The persistent threat of tuberculosis – Why ending TB remains elusive? J Clin Tuber Other Mycobact Dis. 2025; 38: 100510. https://doi.org/10.1016/j.jctube.2025.100510
2. Mbishi, J. V., Chombo, S., Luoga, P., et al. Malaria in under-five children: prevalence and multi-factor analysis of high-risk African countries. BMC Publ Health. 2024; 24 (1):
3. Mohammadnabi, N., Shamseddin, J., Emadi, M., et al. *Mycobacterium tuberculosis*: The Mechanism of Pathogenicity, Immune Responses, and Diagnostic Challenges. J Clin Lab Anal. 2024; 38 (23):
4. Bloom, B. R., Atun, R., Cohen, T., et al. Tuberculosis. In: Holmes, K. K., Bertozzi, S., Bloom, B. R., and Jha, P. (eds). Washington DC: The International Bank for Reconstruction and Development. The World Bank. 3rd edn. 2017. https://www.ncbi.nlm.nih.gov/books/NBK525174/
5. Nguyen, T. N. A., Anton-Le Berre, V., Bañuls, A. L., and Nguyen, T. V. A. Molecular diagnosis of drug-resistant tuberculosis: A literature review. Front Microbiol. 2019; 10: 794. https://doi.org/10.3389/fmicb.2019.00794

6. Amin, M., Afrazeh, E., Seyyedian-Nikjeh, S. F., Meskini, M., Doroud, D., and Siadat, S. D. New insight in molecular detection of *Mycobacterium tuberculosis*. AMB Express. 2024; 14 (1): 74 doi: 10.1186/s13568-024-01730-3
7. Srisuwanvilai, L. O., Monkongdee, P., Podewils, L. J., et al. Performance of the BACTEC MGIT 960 compared with solid media for detection of *Mycobacterium* in Bangkok, Thailand. Diagn Microbiol Infect Dis. 2008; 61 (4): 402–407. https://doi.org/10.1016/j.diagmicrobio.2008.03.018
8. Gavi, K., and Saini, P. Optimizing HIV Testing in Infants: Enhancing Blood Sample Collection, Transport, and Healthcare Infrastructure in Nigeria 2024. https://www.researchgate.net/publication/387169957_Optimizing_HIV_Testing_in_Infants_Enhancing_Blood_Sample_Collection_Transport_and_Healthcare_Infrastructure_in_Nigeria
9. Tessema, B., Beer, J., Emmrich, F., Sack, U., and Rodloff, A. C. Rate of recovery of *Mycobacterium tuberculosis* from frozen acid-fast bacillus smear-positive sputum samples subjected to long-term storage in northwest Ethiopia. J Clin Microbiol. 2011; 49(7): 2557–2561. https://doi.org/10.1128/JCM.02403-10
10. Kwon, B. S., Park, J. S., Shin, J. A., et al. A prospective study on the long-term storage of sputum and the recovery of nontuberculous mycobacteria. Therap Adv Respir Dis. 2023; 17: 17534666231162244. doi: 10.1177/17534666231162244
11. David, A., Scott, L. E., Da Silva, P., Mayne, E., and Stevens, W. S. Storage of *Mycobacterium tuberculosis* culture isolates in Microbank™ beads at a South African laboratory. Afr J Lab Med. 2023; 12 (1): 2172. doi:10.4102/ajlm.v12i1.2172.
12. Lumb, R., Ardian, M., Waramori, G., et al. An alternative method for sputum storage and transport for *Mycobacterium tuberculosis* drug resistance surveys. Int J Tuber Lung Dis. 2006; 10 (2): 172–177. https://doi.org/10.5588/ijtld.05.0196
13. Malamule, D., Moreira, S., Madeira, C., et al. Quality Analysis of Tuberculosis Specimens Transported by Drones versus Ground Transportation. Drones. 2022; 6 (7): 155. https://doi.org/10.3390/drones6070155
14. Azam, K., Cadir, N., Madeira, C., Gillespie, S. H., and Sabiiti, W. OMNIgene. SPUTUM suppresses contaminants while maintaining *Mycobacterium tuberculosis* viability and obviates cold-chain transport. ERJ Open Res. 2018; 4 (1): 00074-2017. doi: 10.1183/23120541.00074-2017
15. Anthony, R., Cobelens, F., Gebhard, A., and van Soolingen, D. Liquid culture for *Mycobacterium tuberculosis*: Proceed, but with caution. Int J Tuber Lung Dis. 2009; 13 (9): 1051–1053. https://doi.org/10.5588/ijtld.09.0156
16. Rivera, A. B., Tupasi, T. E., Grimaldo, E. R., Cardano, R. C., and Co, V. M. Rapid and improved recovery rate of *Mycobacterium tuberculosis* in Mycobacteria Growth Indicator Tube combined with solid Löwenstein-Jensen medium. Int J Tuber Lung Dis. 1997; 1 (5): 454–459. https://doi.org/10.5588/ijtld.97.454
17. Mambuque, E., Saavedra, B., Molina-Moya, B., et al. Evaluation of OMNIgene-Sputum for preservation of sputum samples for diagnosis of *Mycobacterium tuberculosis*. Trop Med Infect Dis. 2023; 8 (7): 367. https://doi.org/10.3390/tropicalmed8070367
18. Campelo, T. A., Cardoso de Sousa, P. R., Nogueira, L., Frota, C. C., and Zuquim Antas, P. R. Revisiting the methods for detecting *Mycobacterium tuberculosis:* what has the new millennium brought thus far? Access Microbiol. 2021; 3 (7): 000245. doi: 10.1099/acmi.0.000245
19. Pagaduan, J. V., and Altawallbeh, G. Advances in TB testing. Adv Clin Chem. 2023; 115: 33–62. https://doi.org/10.1016/bs.acc.2023.02.001
20. Das, D., Dwibedi, B., and Kar, S. K. Transportation of sputum samples in cetylpyridinium chloride for drug resistance studies from remote areas of Odisha, India. J Infect Develop Ctries. 2014; 8(8): 1059–1062. https://doi.org/10.3855/jidc.4350
21. Tagliani, E., Alagna, R., Tafaj, S., Hafizi, H., and Cirillo, D. M. Evaluation of *Mycobacterium tuberculosis* viability in OMNIgene-SPUTUM reagent upon multi-day transport at ambient temperature. BMC Infect Dis. 2017; 17(1): 735. https://doi.org/10.1186/s12879-017-2838-8
22. Selvakumar, N., Gopi, P., Venkataramu, K., Datta, M., Paramasivan, C., and Prabhakar, R. Isolation of tubercle bacilli from sputum samples of patients in field studies by the cetylpyridinium chloride-sodium chloride and sodium hydroxide methods. Indian J Med Res. 1995; 102: 149–151.
23. Ajide, B., Chuku, A., and Orole, K. Prevalence of *Mycobacterium tuberculosis* complex in North central Nigeria. Nig J Microbiol. 2020; 34(2): 5325–5332.
24. Federal Republic of Nigeria. Federal Ministry of Health Department of Public Health. National Tuberculosis, Leprosy and Buruli Ulcer Control Programme National Guidelines on the Implementation of TB Loop-Isothermal Mediated Amplification (TB-LAMP) test for the Diagnosis of Tuberculosis. https://ntblcp.org.ng/content/uploads/2023/06/National-Guidelines-for-the-Implementation-of-TB-Lamp-in-Nigeria-June-2021-1.pdf
25. WHO. Guidance on regulations for the transport of infectious substances. World Health Organization 2023.https://iris.who.int/bitstream/handle/10665/376214/9789240089525-eng.pdf
26. Bradner, L., Robbe-Austerman, S., Beitz, D. C., and Stabel, J. R. Chemical decontamination with N-acetyl-L-cysteine–sodium hydroxide improves recovery of viable *Mycobacterium avium* subsp *paratuberculosis* organisms from cultured milk. J Clin Microbiol. 2013; 51 (7): 2139–2146. https://doi.org/10.1128/JCM.00644-13
27. Selvakumar, N., Rahman, F., Rajasekaran, S., Narayanan, P. R., and Frieden, T. R. Inefficiency of 0.3% Carbol Fuchsin in Ziehl-Neelsen Staining for Detecting Acid-Fast Bacilli. J Clin Microbiol. 2002; 40 (8): 3041–3403. doi: 10.1128/JCM.40.8.3041-3043.2002.
28. SD BIOSENSOR. TB LAM Ag Test Product information. 2021. https://www.sdbiosensor.com/product/product_view?product_no=227 (Accessed Aug 13, 2025).
29. Lam, E., Nateniyom, S., Whitehead, S. J., et al. Use of Drug-Susceptibility Testing for Management of Drug-Resistant Tuberculosis, Thailand, 2004–2008. Emerg Infect Dis. 2014; 400 - 408 doi: 10.3201/eid2003.130951.
30. Musisi, E., Sessolo, A., Kaswabuli, S., et al. High *Mycobacterium tuberculosis* Bacillary Loads Detected by Tuberculosis Molecular Bacterial Load Assay in Patient Stool: a Potential Alternative for Non sputum Diagnosis and Treatment Response Monitoring of Tuberculosis. Microbiol. Spectr. 2022; 10 (1): e0210021. doi: 10.1128/spectrum.02100-21
31. Hargreaves, J. R., Boccia, D., Evans, C. A., Adato, M., Petticrew, M., and Porter, J. D. H. The social determinants of tuberculosis: From evidence to action. Am J Publ Health. 2011; 101 (4): 654–662. https://doi.org/10.2105/AJPH.2010.199505
32. Kassa, G. M., Merid, M. W., Muluneh, A. G., and Fentie, D. T. Sputum smear grading and associated factors among bacteriologically confirmed pulmonary drug-resistant tuberculosis patients in Ethiopia. BMC Infect Dis. 2021; 21(1): 238 doi: 10.1186/s12879-021-05933-y.