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Comparative Analysis Between Genexpert and AAFB Smear technique in *Mycobacterium Tuberculosis* Detection in Sputum Samples from HIV-Negative Patients

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Abstract
The conventional method for Mycobacterium tuberculosis diagnosis has been the AAFB smear microscopy, which is cost-effective but has limited sensitivity and specificity. On the other hand, molecular diagnostic techniques like GeneXpert have emerged, offering higher sensitivity and the additional capability to detect rifampicin resistance. This study aimed to conduct a comparative analysis between GeneXpert assay and AAFB smear microscopy in Mycobacterium tuberculosis detection in sputum specimens from HIV-negative patients. Sputum samples were collected into a sterile container and AFB microscopy was carried out through conventional stained-slide microscopy. Assays by GeneXpert were performed using the manufacturer's guidelines. Data obtained from this research was analyzed using GraphPad Prism version 9.0. Of the 133 samples tested, the number of positives for the AAFB system was 11 (36.7%) while the number of positives for GeneXpert was 19 (63.3%). The males in this study had a higher positive rate than their female counterparts (57.9% for GeneXpert and 63.6% for AAFB). Patients who fell into the 18-27-year age group had the highest positive rate compared to other age groups (52.6% for GeneXpert and 54.5% for AAFB). This study demonstrated the superior sensitivity of the GeneXpert system over AAFB smear microscopy in detecting Mycobacterium tuberculosis among HIV-negative patients. It is recommended that the GeneXpert system be prioritized where possible in tuberculosis diagnostic protocols to improve detection rates.

Keywords: Acid Fast Bacilli smear technique, GeneXpert assay, HIV negative patients, *Mycobacterium tuberculosis*, Sputum

INTRODUCTION
Tuberculosis (TB) is an infectious disease caused by members of the genus Mycobacterium (WHO, 2016). Nine species in the genus mycobacterium are referred to as *Mycobacterium tuberculosis* complex (MTBC). Among the MTBC are *Mycobacterium tuberculosis* (MTB), *M. africanum,* and *M. canetti* which are the common cause of TB in humans. *M. tuberculosis* is an intracellular, obligate-aerobic, non-spore-forming, non-motile, catalase-negative bacteria. The organism has very poor reaction with the Gram stain, as such cannot be called neither Gram-

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positive nor Gram-negative. The weakly positive cells are sometimes detected on Gram stain by a phenomenon called "ghost cells" (Terraciano *et al*., 2020).

Tuberculosis (TB) has become a notable worldwide health challenge because in 2019 alone, an estimated 10 million new cases and 1.4 million deaths were reported. The *M. tuberculosis* organism kills more people than any other infectious agent (Banuls *et al*., 2015). Initially, the worldwide incidence of TB per capita increased in 2003 but seems to have been stable or has begun to decrease. Incidence in 100,000 population was approximately stable in European regions but was falling in 5 other WHO regions. It was also falling in all 9 sub-regions, with the possible exception of African countries with low HIV prevalence (Africa – low HIV) (Chakaya *et al*., 2021). A decrease of 1.5% in TB incidence worldwide was noticed between 2014 and 2015. The country with the highest incidence of TB disease in 2015 was India with an estimated TB burden of 2.84 million (with uncertainty interval of 1.47–4.65 million). Africa with a TB burden of 26% is among the six countries in decreasing order of incidence: by region, 61% of cases were in Asia; 7% in the Eastern Mediterranean; 3% in Europe; and 3% in the Americas with 60% cases of TB. HIV coinfection reported in 11% of cases and was greatest in countries in Southern Africa (Kumar and Kon, 2017).

There were 1.4 million deaths as a result of TB among HIV-negative people (19 per 100,000 population), and 84% of the deaths occurred in Africa and Southeast Asia in the same year and a further 0.39 million deaths from TB occurred among HIV-positive individuals. About 43% of the TB deaths occurred in India and Nigeria among HIV-negative and HIV-positive people combined. Overall, TB mortality rates have been falling in all continents and there has been a 34% reduction in the TB mortality rate between 2000 and 2015 (Chatterjee and Pramanik, 2015). Accurate and rapid diagnosis of TB has been seen as important for effective patient management in the control of the disease. GeneXpert and AFB (acid-fast bacilli) smear microscopy are two frequently used diagnostic methods for *Mycobacterium tuberculosis* detection in sputum specimens. While both methods have their own advantages and limitations, a comprehensive comparative analysis is needed to evaluate their performance characteristics, cost-effectiveness, and impact on patient outcomes (Terracciano *et al*., 2020).
Traditional diagnostic methods, such as sputum smear microscopy for acid-fast bacilli (AFB), have limitations in sensitivity and specificity. These methods can miss a substantial number of TB cases, particularly in patients with low bacterial loads or those co-infected with HIV. The slow and labor-intensive nature of smear microscopy further complicates timely diagnosis and treatment initiation.

The GeneXpert assay is a molecular test that offers rapid and highly sensitive detection of *Mycobacterium tuberculosis* which is critical for managing and treating TB effectively, especially in regions with high rates of TB. This study aims to carry out a comparative analysis between GeneXpert and AFB smear microscopy in *Mycobacterium tuberculosis* detection in sputum specimens from HIV-negative adult patients attending the out-patient clinics in a tertiary health institution located in Benin City, Edo State, Nigeria

MATERIALS AND METHODS

Study Area

This study was performed at the Medical Microbiology Laboratory of the University of Benin Teaching Hospital (UBTH), Benin city, Edo state, Nigeria. UBTH is a healthcare service provider in West Africa with multi-specialty. The hospitals' location is in Ugbowo, Benin City, and it was established May 12th, 1973, due to the enactment of an edict (number 12) of the

Nigeria National Health Act. The UBTH is an 850-bed tertiary healthcare facility with about 3870 personnel and provides referrals in emergency and primary care services to Edo and neighbouring states (Delta, Ondo, Kogi and Anambra states) (Obaseki *et al*., 2021).

Study Design

This is a cross-sectional study conducted to evaluate the diagnostic performance of the AAFB test and GeneXpert assay among HIV-negative patients who presented with clinical symptoms of pulmonary TB and attended outpatient clinics at a tertiary health institution located in Benin City, Edo State, Nigeria.

Ethical Consideration

Ethical approval was obtained from the Health Research Ethics Committee (HREC), University of Benin Teaching Hospital, Benin-City, Edo State, with reference no: ADM/E 22/A/VOL. VII/148381521838.

Sample Collection

The study population included one hundred and thirty-three (133) adult individuals who were clinically diagnosed as TB patients with HIV-negative status. Sixty-six of the participants were males while sixty-seven were females. The criteria for selection of participants included the ability to produce sputum without inducement, being adult patients from 18 years of age, being suspected TB cases based on clinical symptoms, and being HIV-negative. The patients' demographical data, such as sex and age, were obtained from the participant's request form and transferred into a checklist. Sputum samples collected routinely were shared into two parts, with one part stored at 2-4°C prior samples analysis.

Laboratory Analysis

Detection of AFB

With the aid of a sterile application stick, a drop of raw sputum was placed on a grease-free glass slide and spread evenly, then dried, and heat-fixed. The smear was then flooded with strong Carbol fuchsin and heated over a Bunsen burner flame up till the arousal of vapour and allowed to cool. The slide was rinsed with water and flooded with 3% acid alcohol for few seconds and again rinsed with water. Thereafter the smear was counter-stained with methylene blue for few minutes and rinsed with water. Gently, with a dry swab, the back of the slide was blotted dry and allowed to air dry. A drop of immersion oil was placed on the stained smear and viewed under the oil immersion objective lens of the microscope and the findings were recorded (Bhirud *et al*., 2017).

GeneXpert

The GeneXpert assay was performed following the manufacturer's guidelines. Briefly, using a sterile pipette 2 ml of the GeneXpert reagent was added to 1 ml of sputum sample, and then incubated at room temperature for 15 mins. Thereafter, the mixture was agitated twice at 5-minute intervals. Then with the aid of a sterile pipette, the liquefied mixture was transferred into the GeneXpert cartridge and loaded into the GeneXpert instrument. The results were ready within 2 hours and recorded accordingly (Ejeh *et al*., 2018).

Statistical Analysis

Data obtained from this research was presented and analyzed using GraphPad Prism version 9.0, while analysis of variance (ANOVA) was done to compare means, and the results were expressed in Mean Standard error of mean as it may occur. $p<0.05$ was accepted as significant.

RESULTS

Table 1: Prevalence of detected *Mycobacterium tuberculosis* among participants in both the GeneXpert system and the AAFB smear microscopy. Of the 133 samples tested, the number of positives for GeneXpert was 19(63.3%) while the number of positives for the AAFB system was found to be 11(36.7%). The prevalence of positive samples is increased using GeneXpert compared to the AAFB method and this was statistically significant (X^2 =753.39, p<0.001).

Table 1: Prevalence of Detected *Mycobacterium tuberculosis* among participants using GeneXpert and AAFB methods

Method	No. Examined (n=133)	No. Positive (n=30)	% Positive	X^2	p-value
GeneXpert	133	19	63.3	753.39	<0.001
AAFB	133	11	36.7		

Table 2: Results stratified by sex and age for both GeneXpert and AAFB methods. Among males, 66 individuals were examined using the GeneXpert method, resulting in 11 positive cases, constituting 57.9% positivity. The chi-square test did not reveal a significant association between sex and GeneXpert results (χ^2 = 0.607, p = 0.436). In comparison, using the AAFB method, 66 males were examined, with 7 positive cases (68.6% positivity), showing no significant association between sex and AAFB results (χ^2 = 1.155, p = 0.283). Among the 67 females examined, 8 were recorded positive for GeneXpert (42.1% positivity) and 4 cases were found to be positive using the AAFB method (36.4% positivity) which was slightly lower than the males but it was not statistically significant.

For the age group 18-27 years, 37 individuals were examined through GeneXpert, with 10 positive cases (52.6% positivity), which was the highest prevalence. It was followed by 28-37 years and 48-57 years which had positive rates of 15.8% each. >57 and 38-47 years were the lowest (10.5% and 5.3% respectively). However, the chi-square test did not show a statistically significant association between age and GeneXpert results (χ^2 = 7.851, p = 0.097). A similar trend was observed for the AAFB method, with 6 positive cases out of 11 for the 18-27-year-old age group (54.5% positivity), with other age groups pulling lower positive rates. But again, the association between age and AAFB results was not significant (χ^2 = 8.119, p = 0.087).

Table 2: Sociodemographic Parameters of Sex and Age Distribution of Positive Cases, comparing results from GeneXpert and AAFB methods

Variable	No. Examined	GeneXpert (% Positive)	X^2	p-value	AAFB (% Positive)	X^2	p value
Gender							
Male	66	11 (57.9)	0.607	0.436	7 (68.6)	1.155	0.283
Female	67	8 (42.1)			4 (36.4)		
Age							
18-27	37	10 (52.6)	7.851	0.097	6 (54.5)	8.119	0.087
28-37	24	3 (15.8)			2 (18.2)		
38-47	25	1 (5.3)			0 (0.0)		
48-57	23	3 (15.8)			2 (18.2)		
>57	24	2 (10.5)			1 (9.1)		

Table 3: Relationship between clinical symptoms and diagnostic outcomes for GeneXpert and AAFB methods. Notably, 20 individuals who experienced coughing up blood or mucus underwent testing with GeneXpert, resulting in 6 positive cases (31.5% positivity), followed by individuals who experienced weight loss, with 5 out of 28 positive cases (26.3% positivity). Chest pain, cough, tiredness, seizures, and difficulty in breathing had lower prevalence rates

(10.5%, and 5.3% each respectively). However, the chi-square test did not reveal a significant association between the symptom and GeneXpert results ($\chi^2 = 10.707$, $p = 0.152$). Similarly, for the AAFB method, 20 individuals with the same symptom were tested, resulting in 3 positive cases (27.3% positivity) as well as individuals who experienced weight loss, with 3 out of 28 positive cases (27.3% positivity). The positive rates for the other symptoms were slightly lower. The association between the symptom and AAFB results was significant ($\chi^2 = 18.592$, $p = 0.010$).

Table 3. Relationship between clinical symptoms and diagnostic outcomes for GeneXpert and AAFB methods.

Clinical Information	No. Examined	GeneXpert Positive (% Positive)	X^2	p value	AAFB Positive (% Positive)	X^2	p value
Difficulty in breathing	2	1 (5.3)	10.707	0.152	1 (9.0)	18.592	0.010
Chest pain	10	2 (10.5)			2 (18.2)		
Weight loss	28	5 (26.3)			3 (27.3)		
Cough	11	1 (5.3)			-		
Cough up blood or mucus	20	6 (31.5)			3 (27.3)		
Fever	29	2 (10.5)			2 (18.2)		
Tiredness	27	1 (5.3)			-		
Seizures	6	1 (5.3)			-		
Total	133	19 (100)			11 (100)		

Table 4: Distribution of positive cases across different clinics/wards, comparing outcomes between GeneXpert and AAFB methods. Notably, at the DOT clinic, 72 individuals underwent testing with GeneXpert, resulting in 13 positive cases (68.4% positivity), while for the AAFB method, 8 positive cases were identified out of 11 individuals that tested positive (72.7% positivity). However, the chi-square test reveals a significant association between the clinic/ward and diagnostic results for either method (GeneXpert: $\chi^2 = 2.021$, $p = 0.568$; AAFB: $\chi^2 = 2.418$, $p = 0.490$).

Table 4: Distribution of positive cases across different wards, comparing outcomes between GeneXpert and AAFB methods

Wards	No. Examined	Positive GeneXpert (%)	X^2	p value	Positive AAFB (%)	X^2	p value
COPD	53	05 (26.3)	2.021	0.568	03 (27.3)	2.418	0.490
A1	07	01 (5.3)			00 (0.0)		
A3	01	00 (0.0)			00 (0.0)		
DOT	72	13 (68.4)			08 (72.7)		
Total	133	19 (100)			11 (100)		

DISCUSSION

Tuberculosis caused by the bacterium *Mycobacterium tuberculosis*, remains a global public health challenge, particularly in regions with a high *Mycobacterium tuberculosis* burden but limited resources (Gaur *et al*., 2020). Despite advancements in diagnostic technologies, the early and accurate detection of *Mycobacterium tuberculosis* cases, especially in HIV-negative individuals, is critical for effective treatment and control of the disease (Nadeem *et al*., 2022). The conventional method for *Mycobacterium tuberculosis* diagnosis has been the AFB smear microscopy, which, while cost-effective and rapid, has limitations in sensitivity and specificity. On the other hand, molecular diagnostic techniques like GeneXpert have emerged, offering higher sensitivity and the additional capability to detect rifampicin resistance (Nadeem *et al*., 2022).

The GeneXpert system has demonstrated a higher prevalence rate of detected *Mycobacterium tuberculosis* in sputum samples compared to the AAFB smear microscopy among the HIV-negative participants studied. This implies that GeneXpert can detect *Mycobacterium tuberculosis* cases that may be missed by AAFB smear microscopy. Comparatively, the findings in this study are in corroboration with studies by Ejeh *et al*. (2018), Sorsa and Kaso, (2021), and a study by Nadeem *et al*. (2022) with all studies demonstrating the superior performance of molecular diagnostics like GeneXpert over traditional methods like AAFB smear microscopy in terms of sensitivity and specificity. The possible reasons for these results include GeneXpert's ability to detect low levels of bacterial DNA, making it more sensitive in identifying TB cases with low bacterial loads. Its molecular basis allows it to bypass some limitations of AAFB smear microscopy, such as the subjective interpretation of smears and the requirement for a higher bacterial load for detection. These advantages make GeneXpert a more reliable and efficient diagnostic tool, particularly in settings with high TB burden and limited diagnostic infrastructure.

The data presented in Tables 2, 3 and 4 offer a multifaceted view of the diagnostic outcomes of GeneXpert and AAFB smear microscopy for detecting *Mycobacterium tuberculosis*, stratified by sex, age, clinical symptoms, and clinic/ward location. The results indicate no significant association between sex and diagnostic outcomes for both GeneXpert and AAFB methods. While GeneXpert showed a slightly higher positivity rate among males (57.9%) compared to AAFB (42.1%), the differences were not significant statistically. This suggests that the diagnostic efficacy of both methods is consistent across sexes, and sex does not significantly influence the likelihood of a positive *Mycobacterium tuberculosis* diagnosis using these methods. This is also reported in most studies utilizing the GeneXpert technique (Habous *et al*., 2019; Magar *et al*., 2020)

The analysis by age group reveals a notably high positivity rate in the 18-27 age group for both GeneXpert (52.6%) and AAFB (54.5%) methods. However, the absence of a statistically significant association between age and diagnostic outcomes suggests that while tuberculosis may be more prevalent in this age group, the diagnostic performance of GeneXpert and AAFB does not vary significantly with age. The high positivity rates could reflect a higher burden of tuberculosis among younger individuals in the study population which was also observed in previous reports (Masab *et al*., 2020; Maya *et al*., 2023).

The relationship between the symptom of coughing up blood or mucus and diagnostic outcomes presents an interesting contrast. While both methods showed a similar positivity rate for this symptom (GeneXpert - 31.5%; AAFB - 27.3%), only the association with AAFB results was statistically significant. This finding reveals the potential relevance of specific clinical symptoms in guiding the choice of diagnostic methods. This was also demonstrated by Ullah *et al*. (2020) suggesting that AAFB smear microscopy may still hold value in cases presenting with classic tuberculosis symptoms, despite its overall lower sensitivity compared to GeneXpert. The analysis by clinics/wards showed a high positive rate in the DOT clinic (GeneXpert: 68.4%, AAFB: 72.7%). The consistent diagnostic performance of GeneXpert and AAFB across different clinics/wards supports the potential for these methods to be broadly implemented in diverse clinical settings.

CONCLUSION

This study has demonstrated the higher sensitivity of the GeneXpert system over AAFB smear microscopy in detecting *Mycobacterium tuberculosis* among HIV-negative patients. Despite the

absence of significant associations between diagnostic outcomes and demographic factors such as sex and age, the findings highlight the importance of employing sensitive diagnostic tools in the fight against *Mycobacterium tuberculosis*. The variability in detection rates based on clinical symptoms and across different clinical settings underscores the complexity of tuberculosis diagnosis and the need for a multifaceted approach. The study reinforces the critical role of advanced molecular diagnostic methods in enhancing *Mycobacterium tuberculosis* control efforts, particularly in settings with a high burden of disease.

Conflict Of Interest

The authors wish to state that they have no conflicting interest in relation to this publication. All research assay was conducted independently, and there was no funding or support received from any organization or entity that could influence the study's results or interpretation.

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REFERENCES

Banuls, A. L., Sanou, A., Van Anh, N. T. and Godreuil, S. (2015). Mycobacterium tuberculosis: ecology and evolution of a human bacterium. *Journal of Medical Microbiology*. **64**(11): 1261-1269.

Bhirud. P., Joshi, A., Hirani, N. and Chowdhary, A. (2017). Rapid laboratory diagnosis of pulmonary tuberculosis. *International Journal of Microbiology* **6**: 296-301.

Chakaya, J., Khan, M., Ntoumi, F., Aklillu, E., Fatima, R., Mwaba, P. and Zumla, A. (2021). Global Tuberculosis Report 2020–Reflections on the Global TB burden, treatment and prevention efforts. *International Journal of Infectious Diseases*. **113**: S7-S12.

Chatterjee, D. and Pramanik, A. K. (2015). Tuberculosis in the African continent: A comprehensive review. *Pathophysiology*. **22**(1): 73-83.

Ejeh, E. F., Undiandeye, A., Akinseye, V. O., Okon, K. O., Kazeem, H. M., Kudi, C. A. and Cadmus, S. I. B. (2018). Diagnostic performance of GeneXpert and Ziehl-Neelson microscopy in the detection of tuberculosis in Benue State, Nigeria. *Alexandria Journal of Medicine*. **54**(4): 529-533.

Gaur, V., Singh, A. K., Kumar, A. and Mishra, A. K. (2020). Utility and effectiveness of genexpert over conventional methods for diagnosis of pulmonary tuberculosis. *International Journal of Pharmaceutical Science and Research*. **11**(9): 4629-4636.

Habous, M., Elimam, M. A. E., Kumar, R. and Deesi, Z. A. (2019). Evaluation of GeneXpert Mycobacterium tuberculosis/Rifampin for the detection of Mycobacterium tuberculosis complex and rifampicin resistance in non-respiratory clinical specimens. *The International Journal of Mycobacteriology*. **8**(2): 132-137.

Kumar, K. and Kon, O. M. (2017). Diagnosis and treatment of tuberculosis: latest developments and future priorities. *Annals of Research Hospitals* **1**: 37

Magar, B. P., Thapa, N., Rana, E., Joshi, K. R., Maharjan, N. and Bista, B. (2020). Prevalence of Tuberculosis by GeneXpert Method in Karnali Academy of Health Sciences Teaching Hospital, Jumla, Nepal. *Biomedical Sciences*. **6**(3): 56-60.

Masab, U., Shajee, A. S. and Muhammad, A. F. (2020). Diagnostic accuracy of sputum microscopy in comparison with GeneXpert In Pulmonary tuberculosis. *Cureus* **12**(11): e11383

Maya, R., Ganesh, P. N., Saraswati, L. and Bodh, B. K. (2023). GeneXpert and acid fast bacilli smear for diagnosis of pulmonary tuberculosis in tertiary care center of rural Nepal. *Nepal Medical Journal*. **5**(10): 11-15

Nadeem, Z., Iqbal, J., Kausar, S., Noor, S., Saleem, I., Munir, N. and Gasmi, A. (2022). Comparative Analysis of the Efficacies of the GeneXpert and Solid Culture Media Techniques in the Diagnosis of Mycobacterium Tuberculosis. *Archives of Razi Institute*. **77**(6): 2053-2060.

Obaseki, D. E., Osaigbovo, I. I., Ogboghodo, E. O., Adeleye, O., Akoria, O. A., Oko-Oboh, G. A., Okwara, B. U., Omuemu, C. E. and Okugbo, S. (2021). Preparedness and response of a tertiary hospital to the COVID-19 pandemic in Nigeria: challenges, opportunities and lessons. *Transactions of The Royal Society of Tropical Medicine and Hygiene*. **115**(7): 727-730.

Sorsa, A. and Kaso, M. (2021). Diagnostic performance of GeneXpert in tuberculosis-HIV co-infected patients at Asella Teaching and Referral Hospital, Southeastern Ethiopia: A cross sectional study. *PloS One*. **16**(1): e0242205.

Terracciano, E., Amadori, F., Zarrati, L. and Franco, E. (2020), Jan-Feb. Tuberculosis: an ever-present disease but difficult to prevent. *Igiene e Sanita pubblica.* **76**(1): 59-66.

Ullah, R., Khan, I., Fahad, M. S., Irfan, M. and Aziz, K. (2020). Diagnostic accuracy of GeneXpert MTB/RIF and AFB smear compared to culture for the detection of Tuberculosis using bronchio-alveolar lavage (BAL) fluid. *Pakistan Journal of Chest Medicine*. **26**(2): 68-73.