Content available at: https://www.ipinnovative.com/open-access-journals

IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: https://www.ijmmtd.org/

Original Research Article

Performance of acid fast techniques and genexpert in diagnosing pulmonary tuberculosis in Uasin Gishu county health facilities, Kenya

Hellen Jeruto Sawe[1,*], Sabella J Kiprono[2], Tom Were[2]

[1] *Dept. of Medical Laboratory Sciences, Masinde Muliro University of Science and Technology, Kenya and Ministry of Health Uasin Gishu County, Kenya*
[2] *Medical Laboratory, Science/Masinde Muliro University of Science and Technology, Kakamega, Kenya*

ARTICLE INFO

Article history:
Received 29-06-2021
Accepted 10-08-2021
Available online 01-09-2021

Keywords:
Performance
Tuberclosis
GeneXpert
Culture
Sensitivity
Specificity
Acid fast techniques

ABSTRACT

Tuberculosis is one of the major causes of death worldwide, millions of people have been infected with pulmonary tuberculosis and over 95% of tuberculosis deaths occur in low and middle income countries as per World Health Organization report. The study assessed the performance of Ziehl Neelsen technique and GeneXpert used for diagnosis of Pulmonary tuberculosis in Uasin Gishu county health facilities, Kenya, between November 2019 and February 2020. A cross-sectional study was conducted in three selected health facilities and a total of 104 participants were enrolled into the study. Sputum samples from suspected tuberculosis patients were tested using Ziehl Neelsen technique, GeneXpert and culture method was used as gold standard. Performance of the tests was done by considering their sensitivity, specificity, predictive values. The results indicated that among the 104 sputum samples tested Ziehl Neelseen technique used detected 23(22.1%) bacilli from spot samples and 33(31.7%) bacilli from morning sputum samples. GeneXpert test detected 49 (47.1%) bacilli. The results also revealed that there was significant difference between Ziehl Neelsen technique using spot samples and GeneXpert and culture test (p-value< 0.05) and Ziehl Neelsen technique using morning test done with culture test had no significant difference (p-value>0.05). Performance of tests should be done accurately for proper diagnosis of TB, GeneXpert should always be used since its sensitivity is high and also detects Rifampicin resistance. Also, Performance of tests should be done using standard procedures to avoid errors in the test results.

1. Introduction

Pulmonary tuberculosis is a communicable disease that is caused by bacterium called Mycobacterium tuberculosis. The disease is airborne affecting the lungs and can also affect other parts of the body. It was estimated globally that there were 9.27 million incident cases of TB,13.7 million prevalent cases,1.32 million deaths TB in HIV negative and 0.45 million deaths in HIV positive persons (WHO, 2018).[1]

Tuberculosis burden in Africa is high, this is compounded by weak health care systems, inadequate laboratories and conditions that promote transmission of infection, this has worsened by the emergence of drug resistant strains (Varma, 2018).[2] Kenya is one of the 30 high burden countries that together account for more than 80% of World TB cases. Kenya faces a triple burden of Human Immunodeficiency Virus and tuberculosis, while tuberculosis remains the fourth highest cause of death among infectious diseases (Kinara, 2016).[3]

Diagnosis of pulmonary tuberculosis involves Microscopic examination of smears in Acid Fast staining techniques which are simple, cheap, quick and effective method for developing countries. Ziehl Neelsen technique is an acid fast technique and is the most widely available

* *Corresponding author.*
E-mail address: sawehellen@gmail.com (H. J. Sawe).

https://doi.org/10.18231/j.ijmmtd.2021.035

test for diagnosing tuberculosis in resource limited settings. Ziehl Neelsen(ZN) smear microscopy is highly specific but its sensitivity is variable (20-80%). To achieve maximum sensitivity of this diagnostic test, it is essential to have a good quality sputum sample that is the sputum should contain mucoid or mucopurulent material and the volume should be at least 3mls (Singhal et al, 2015).[4]

GeneXpert has a higher sensitivity than Acid Fast smear microscopy in respiratory samples. It can be a useful tool for easy diagnosis of patients with high clinical suspicion of pulmonary tuberculosis and also for detection of multidrug resistance (CDC,2009).[5] GeneXpert test is a useful diagnostic method in suspected pulmonary tuberculosis cases either Acid Fast smear negative or positive due to its rapidity and detection of rifampicin resistance which is beneficial to patients with Multi-Drug Resistance and Human Immunodeficiency Virus associated tuberculosis (Alcaide et al, 2011).[6]

Effective treatment, management and control of tuberculosis in endemic areas depends on accurate diagnosis of the tuberculosis which can be achieved by adherence to the standard operating procedures of the available techniques. Therefore the study aims at assessing the performance of Acid Fast staining techniques and GeneXpert test as used for diagnosis of pulmonary tuberculosis in Uasin Gishu county health facilities.

2. Materials and Methods

2.1. Study site

The study was conducted in Uasin Gishu county selected health facilities (Uasin Gishu county hospital, Huruma subcounty hospital and Moi Teaching and Referral hospital) which are situated within Eldoret town. Uasin Gishu county has a population of 1,163,180 with an area of 3392Km^2 The county have one county hospital, six subcounty hospitals, 33 health centres and 88 dispensaries and also has one Referral hospital. Patients from the subcounty hospitals and dispensaries are referred to the selected health facilities for further management. The health facilities serve majority of the tuberculosis infected patients who lives within the county since tuberculosis is among the top diseases causing morbidity and mortality within the region.

2.2. Study design

A cross sectional study design was used, where 104 sputum samples (both spot and morning) from suspected TB patients were tested and performance of ZN technique and GeneXpert was done Using culture test as Gold standard. The results were interpreted and documented. Data collected from the results obtained was used to determine the performance of the tests done with culture test as the gold standard. Identification of factors affecting the performance of Ziehl Neelsen technique and GeneXpert as done by laboratory personnel was done.

2.3. Study population

The target population included all suspected TB patients between the age of 15 and 70 years who presented with signs and symptoms for tuberculosis and had already given their consent for the study. These patients were all sent to the Laboratory for tuberculosis screening using the three tests, Ziehl Neelsen technique, GeneXpert and culture test.

2.4. Sampling technique

The health facilities were selected using purposive sampling technique. Suspected TB patients were grouped according to age categories and gender as they attended laboratory for TB testing.

The suspected patients who were aged between 15 and 70 years were considered for the study. The study employed a cross-sectional study design. This design involved collecting and testing samples from suspected TB patients and data collected from the results obtained. The performance of the tests was done with culture test used as gold standard.

2.5. Sample size calculation

The sample size calculation was done by use of Yamane formula (Kasiulevicius, 2006).[7] A total of 104 suspected TB patients participated in the study. The calculation from the population of patients who attended the three selected health facilities in Uasin Gishu county led to the right sample size to be used.

2.6. Ethics

The study was approved by the supervisors and the school of Graduate studies Masinde Muliro University of Science and Technology. Permission for the study was sought from Masinde Muliro University of Science and Technology Ethical and Research committee and National Commission for Science and Technology. Permission was also sought from the hospital management of Moi Teaching and Referral Hospital, Uasin Gishu county hospital and Huruma subcounty hospital, which also included Permission from the Laboratory managers and personnel from the three facilities. The proposal was forwarded to the three facilities ethical review committee for approval to conduct the study.

The patients were counseled by medical personnel and allowed to make their decisions and consent was obtained from each suspected patient prior to enrolling them into the study. Consent from the minor group was obtained from their parents and guardians. Consent from the patients concerning their sputum samples being used for research assured the patients of the confidentiality of their results and continuation of their treatment without interference.

The benefits of the study like obtaining proper diagnosis and being given the right treatment was explained to the participants prior to data collection. All Participants were assured that samples were collected by well trained, experienced and qualified personnel.

2.7. Inclusion criteria

Patients aged between 15 and 70 years suspected to have pulmonary tuberculosis with symptoms including cough for two weeks or more, weight loss, fatigue, fever, loss of appetite and night sweats and have been referred from chest clinic to the laboratory for Acid Fast microscopy and GeneXpert test. Laboratory personnel carrying out TB tests in the selected health facilities were also included in the study.

2.8. Exclusion criteria

Patients who were suspected to have tuberculosis but refused to consent and also those who were below the age of 15 or above 70 years were not included in the study. Laboratory personnel who were not participating in TB testing were excluded from the study.

2.9. Sample collection

Sputum containers were used for sputum sample collection, two sputum samples were collected. The first sample was collected on the spot at the consultation when the patient was identified as suspected tuberculosis case. The second sample was collected early in the morning, right after the patient woke up and before eating. Spot and morning sputum samples were tested using Ziehl Neelsen technique, GeneXpert and culture test. Results obtained after testing was reported and recorded on tuberculosis registers, data was obtained from the results in the registers.

2.10. Performance of ZN technique using Culture method as gold standard.

Sputum samples collected was processed by laboratory personnel who had undergone competency assessment test on procedures done for diagnosis of tuberculosis. The laboratory personnel ensured presence of Standard Operating Procedures for preparations of reagents and also availability of equipments maintenance logs before carrying out procedures for tuberculosis tests, confirmation of the reagents quality and samples sufficiency before testing was done. The procedure for preparation of sputum smears, staining and microscopic examination was done as per the standard procedures. After testing, examination of smears was done and the results were reported. Smear negative was indicated as No Acid Fast Bacilli seen after examining 300 fields in the smear. Any red bacilli seen, the smear was reported as Acid Fast Bacilli positive and the number of bacteria present was indicated. The results were reviewed before being dispatched. Culture method was used as the gold standard. Culture was done by growing organisms on culture media (Lowenstein Jensen medium) under the required temperatures and growth of Mycobacterium tuberculosis (MTB) took a longer period between 6 to 8 weeks.

2.11. Performance of GeneXpert test using Culture method as gold standard

The sputum samples collected was mixed with the reagent that was provided with the assay, and a cartridge containing this, placed in the GeneXpert machine and all processing from this point was fully automated. Interpretation of GeneXpert results was as follows**;** GeneXpert was interpreted along with clinical, radiographic, and other laboratory findings. Results from the GeneXpert assay indicated whether or not Mycobacterium tuberculosis was detected in the sample. In some instances where the results was found to be invalid, the test was repeated. When Mycobacterium tuberculosis was detected, the results also states whether there was resistance, testing for drug resistance to both first-line and second-line drugs should then be performed whenever there is drug resistance so that an effective treatment regime can be selected.

2.12. Data analysis

Statistical analysis was conducted with statistical package for social science (SPSS) version 20.0. The sensitivity, specificity, positive predictive value and negative predictive value of Ziehl Neelsen technique and GeneXpert test was calculated, culture method was used as the gold standard. Data generated from the three tests was entered into Microsoft excel and exposed to SPSS to establish the relationship between the results. MacNemar test was used to demonstrate relationship between the results obtained. The data obtained from Ziehl Neelsen technique and GeneXpert test was done with culture method as the gold standard and data was presented using tables.

3. Results

3.1. Demographic characteristics of suspected TB patients

The demographic characteristics of suspected tuberculosis patients are summarized in table below. A total of 104 suspected tuberculosis patients were enrolled in the study and categorized according to age, gender, level of education, marital status, occupation and Body mass index levels.

Data presented are numbers (n) and proportions (%) of suspected TB patients. Variables of age, gender, level of education, marital status, occupation and Body Mass Index. Others; includes street people and house helps.Table 1

Table 1: Demographic characteristics of suspected TBpatients

Variables	Characteristics	n (%)
Median age (range), years		32 (15-69)
Gender	Male	60 (57.7%)
	Female	44 (42.3%)
Level of education	Primary	28 (26.9%)
	Secondary	39 (37.5%)
	Tertiary	37 (35.6%)
Marital status	Married	69 (66.3%)
	Single	24 (23.1%)
	Widowed	8 (7.7%)
	Divorced	3 (2.9%)
Occupation	Government sponsored	9 (8.7%)
	Self-sponsored	60 (57.7%)
	Students	13 (12.4%)
	Others	22 (21.2%)
Body Mass Index (BMI) (First visit)	Underweight	30 (28.8%)
	Normal	57 (54.8%)
	Overweight	4 (3.8%)
	Obese	3 (2.9%)
Body Mass Index (BMI) (Second visit)	Underweight	46(44.2%)
	Normal	51(49.0%)
	Overweight	5(4.8%)
	Obese	2(1.9%)

BMI is body mass index.BMI ≤18.5 underweight, 18.5-24.9 normal ≥25.0-29.9 overweight, ≥30 obese. Age is presented as median range in years. Level of education, marital status, occupation and BMI are presented as number (n), and proportion (%) of suspected patients. Others; includes street people and house helps.

3.2. Performance of ZN technique (spot test) using culture test as Gold standard

Performance of Ziehl Neelsen technique using spot sputum samples was done using culture test as the gold standard and 104 samples tested. MacNemar test was used to test the difference in the Ziehl Neelsen (spot test) done with culture as the gold standard.

Data presented are numbers and proportions (%), of ZN (spot test) and culture test. Values and 95% confident intervals of Sensitivity, specificity and Predictive Values. McNemar(p-value) was also presented.Table 2

3.3. Performance of Ziehl Neelsen technique (Morning test) using culture test as Gold Standard

Performance of Ziehl Neelsen technique using sputum Morning samples was done and culture test which was used as the gold standard. MacNemar test was used to test the difference in the Ziehl Neelsen test done the gold standard.

Data presented are numbers and proportions (%) of ZN (Morning test) with culture test as Gold standard. Values and 95% confident intervals of Sensitivity, specificity, Predictive Values were presented. McNemar (P-value) and also presented.Table 3

3.4. Performance of GeneXpert test using Culture test as gold standard.

Performance of GeneXpert test was done using culture test which was used as the gold standard. MacNemar test was used to test the difference in the GeneXpert test done using culture test as a gold standard. GeneXpert test was done with culture test in which 104 sputum samples was also tested.

Data presented are numbers and proportions (%) of (GeneXpert test) using culture test as Gold standard. Values and 95% confident intervals of Sensitivity, specificity and Predictive Values. McNemar (P-value) also presented.Table 4

4. Discussion

Performance of Ziehl Neelsen technique (ZN) and culture test was assessed in this study. The suspected tuberculosis patients were tested using Ziehl Neelsen technique, both spot and morning samples were collected and tested. ZN (morning test) detected more Acid Fast Bacilli(AFB) positives than ZN(spot test). Culture test was used as a gold standard where culture positive was indicated as Mycobacterium tuberculosis(MTB) isolated,and culture negative was indicated as MTB not isolated. The sensitivity of Ziehl Neelsen-Morning test was higher than Ziehl Neelsen-Spot test and the same trend for their Negative Predictive Values. The specificity of ZN- Morning test was lower than specificity for ZN-Spot test and same trend for their Positive Predictive Values. This indicated that ZN-Morning test was more effective in detecting TB in the suspected patients. According to the study ZN -Spot test showed few bacilli compared to ZN-Morning test which showed more bacilli. Early morning samples contained mucoid or mucopurulent material while most spot samples were salivary. However, there was no significant difference between Ziehl Nielsen technique (morning test) and Culture test (0.6636).

The sensitivity of Ziehl Nielsen-Morning test in the present study was (66.67%) almost close to (61.1%) reported from South Africa, Cape Town (Marais, 2008).[8] This might have result from the sample size (221) in the study of Cape Town. The specificity of Ziehl Nielsen-Spot test (94.12%) was almost same as the study conducted in Pakistan showing specificity (91.4%) (Munir et al, 2015) and the difference might be attributed to sample size.

The performance of GeneXpert test was also assessed in the study against the culture test which was used as a gold standard. Sputum samples were processed using GeneXpert test which detected both positive and negative results. Culture test was also done to confirm the test results. GeneXpert and culture test done showed higher sensitivity and this implied that, GeneXpert has been reported to have more sensitivity than Acid fast techniques due to its rapidity and detection of Rifampicin resistance (Agrawal et

Table 2: Showing Performance of Ziehl Neelsen technique (spot test) using culture test as Gold standard.

		MTB Isolated	MTB not isolated	n	(%)	P-value
	Positive	19	4	23	22	0.0072
ZN(Spot test)	Negative	17	64	81	78	
	n (%)	36	68	104	100	
		Values (%)				95% CI
Sensitivity		52.78				35.50-69.63
Specificity		94.12				85.61- 98.37
Positive Predictive Value		82.61				61.19- 95.05
Negative Predictive Value		79.01				68.58- 87.27

Table 3: Showing Performance of Ziehl Neelsen technique (Morning test) with culture test used as Gold standard.

		MTB isolated	MTB not isolated	n	(%)	P-value
	Positive	24	9	33	32	0.6636
ZN(Morning test)	Negative	12	59	71	68	
	n (%)	36	68	104	100	
		Values (%)				95% CI
Sensitivity		66.67				49.05- 81.43
Specificity		87.76				76.38- 93.76
PPV		72.73				54.50- 86.70
NPV		83.10				72.31- 90.96

Table 4: Showing Performance of GeneXpert test using culture test as Gold standard.

		MTB isolated	MTB not isolated	n	(%)	P-Value
GeneXpert	Positive	32	17	49	47	0.0072
	Negative	4	51	55	53	
	n (%)	36	68	104	100	
		Values (%)				95% CI
Sensitivity		88.89				73.96- 96.89
Specificity		75.00				63.05- 84.69
PPV		65.31				50.34- 78.34
NPV		92.73				82.42- 97.98

al, 2016).[9] GeneXpert test had a low specificity (75.0%).

The sensitivity of GeneXpert in the present study was almost similar to study conducted in other countries such as India and Pakistan with sensitivity of at least 86.8% and a maximum of 90.1%. (Lombardi, 2017).[10] In this study, GeneXpert showed higher sensitivity of 88.89%, which was similar to the sensitivity of GeneXpert in a study done in Addis Ababa, Ethiopia (Taddesse et al, 2018).[11] GeneXpert and AFB microscopy share almost same specificity but sensitivity of GeneXpert is much higher.

5. Recommendations

The laboratory personnel carrying out procedures for Ziehl Neelsen technique for diagnosis of tuberculosis should always process the sputum samples according to the standard operating procedures. GeneXpert test should always be used in diagnosis of tuberculosis since its sensitivity is high and also it detects Rifampicin resistance, Culture test should always be used a gold standard.

6. Limitation of the study

This acid fast technique used in this study is commonly used for diagnosis of pulmonary TB but does not distinguish between viable and dead organisms, it also has a limited sensitivity since it requires a high bacterial load. GeneXpert test has a high cost, it needs a high electricity and annual calibration. Culture media as used in this study is a gold standard but it takes a longer period for growth of organisms.

7. Conclusion

Ziehl Neelsen Technique using morning sputum samples has higher sensitivity than Ziehl Neelsen technique with the use of spot sputum samples. Performance of GeneXpert test showed high sensitivity and Culture test takes a longer period for the growth of Mycobacterium tuberclosis but

culture is considered a gold standard.

8. Acknowledgements

The study participants who consented for the study making it possible, my supervisors (Masinde Muliro University of Science and Technology) for their good support that has led to the success of this study. The management of Uasin Gishu County health facilities, for fully participating in identifying participants and guiding on the suspected cases, the Medical laboratory personnel for offering full support during the study.

9. Conflict of Interest

The authors have no any conflict of interest

10. Source of Funding

The Authors did not receive any grant for the study. The expenses for the study were funded by the corresponded author.

References

1. World Health Organization. Compendium of WHO guidelines and associated standards: ensuring optimum delivery of the cascade of care for patients with tuberculosis. In: 2nd Edn.; 2018. Available from: https://apps.who.int/iris/handle/10665/272644.
2. Varma JK, Oppong-Otoo J, Ondoa P, Perovic O, Park BJ, Laxminarayan R, et al. Africa Centres for Disease Control and Prevention's framework for antimicrobial resistance control in Africa. *Afr J Lab Med*. 2018;7(2):1–4.
3. Kinara J. Influence Of Health Information Application System On Service Delivery To Patients In Moi Teaching &referral Hospital, Uasin Gishu County, Kenya; 2016. Available from: http://hdl.handle.net/11295/98694.
4. Singhal R, Myneedu VP. Microscopy as a diagnostic tool in pulmonary tuberculosis. *Int J Mycobacteriol*. 2015;4(1):1–6. doi:10.1016/j.ijmyco.2014.12.006.
5. Updated guidelines for the use of nucleic Kinara J. Influence Of Health Information Application System On Service Delivery To Patients In Moi Teaching &referral Hospital. *MMWR Morbidity and mortality weekly report*. 2009;58(1):7–7.
6. Alcaide F, Coll P. Advances in rapid diagnosis of tuberculosis disease and anti-tuberculous drug resistance. *Enferm Infecc Microbiol Clin*. 2011;29(1):34–40. doi:10.1016/S0213-005X(11)70016-7.
7. Kasiulevičius V, Šapoka V, e RF. Sample size calculation in epidemiological studies. *Gerontologija*. 2006;7(4):225–31.
8. Marais BJ, Brittle W, Painczyk K, Hesseling AC, Beyers N, Wasserman E, et al. Use of light-emitting diode fluorescence microscopy to detect acid-fast bacilli in sputum. *Clin Infect Dis*. 2008;47(2):203–7.
9. Agrawal M, Bajaj A, Bhatia V, Dutt S. Comparative study of GeneXpert with ZN stain and culture in samples of suspected pulmonary tuberculosis. *J Clin Diagn Res: JCDR*. 2016;10(5):9.
10. Lombardi G, Gregori VD, Girometti N, Tadolini M, Bisognin F, Monte PD, et al. Diagnosis of smear-negative tuberculosis is greatly improved by Xpert MTB/RIF. *PloS One*. 2017;12(4):e0176186. doi:10.1371/journal.pone.0176186.
11. Taddee BD, Desalegn DM, Misganaw AS, Kitila KT, Hailu TK, Bika AT, et al. Diagnostic Performance of Xpert MTB/RIF Assay Versus Ziehl-Neelsen Method for the Diagnosis of Pulmonary Tuberculosis in Addis Ababa, Ethiopia. *Open Microbiol J*. 2018;12:390–6. doi:10.2174/1874285801812010390.

Author biography

Hellen Jeruto Sawe, Medical Laboratory Officer

Sabella J Kiprono, Senior Lecturer

Tom Were, Senior Lecturer

Cite this article: Sawe HJ, Kiprono SJ, Were T. Performance of acid fast techniques and genexpert in diagnosing pulmonary tuberculosis in Uasin Gishu county health facilities, Kenya. *IP Int J Med Microbiol Trop Dis* 2021;7(3):165-170.