

#### How to Cite

Cruz, R. da, Silva, H. da, & Alves, L. (2024). Detection of mycobacterium tuberculosis by microscopic examination and gene expert in a suspected patient with pulmonary tuberculosis disease, in Dili City, East Timor. *International Journal of Health & Medical Sciences*, 7(1), 11-16. <https://doi.org/10.21744/ijhms.v7n1.2238>

# Detection of Mycobacterium Tuberculosis by Microscopic Examination and Gene Expert in a Suspected Patient with Pulmonary Tuberculosis Disease, in Dili City, East Timor

**Renato da Cruz**

*Universidad da PAZ, Timor-Leste*

**Hendriketa da Silva**

*Universidad Nacional Timor Lorosa'e, Timor-Leste*

*Corresponding author email: [dasilvahendriketa@gmail.com](mailto:dasilvahendriketa@gmail.com)*

**Liborio Alves**

*Universidad da PAZ, Timor-Leste*

**Abstract---***Tuberculosis is one of the most dangerous and deadly diseases, so control is needed to reduce the transmission rate of TB disease and mortality among the community, so the gene method is very appropriate to be able to help in diagnosing TB. Acid-resistant bacilli are bacteria that have a very thick lipid content so that the staining cannot be affected by other dye reactions, except by using phenol and through the heating process. Mycobacterium tuberculosis has a cell wall consisting of a waxy coating and mycolic acid; therefore, these bacteria can only color with BTA. Mycobacterium Tuberculosis can maintain the first color, namely Carbin Fuchin, and fight decolorization with alcoholic acid, so this bacterium is called an acid-resistant bacillus. The right examination method to diagnose TB disease is necessary to be able to follow the appropriate treatment so as to reduce transmission and death rates due to TB disease. The aim of this research is to analyze the difference in examination results using microscopes and Gene Xpert machines in Dili district in suspected patients with pulmonary tuberculosis. The method of research is experimental research with a cross-sectional study approach (cross-sectional study), with a total sample of 171 using sampling techniques; nonprobability sampling was taken asidental using data processing techniques with the SSPS system; and analysis techniques using univariate and bivariate. The research location was Dili Municipality, and the research time was from April to June 2022. Research Results: using a microscope with the Zeel Nelsen method, negative examination results as many as 152, scanty 6 samples, 1+, ten positive casein 2+, two samples, 3+. For gene Xpert, the Catridge method identified MTB not detected as many as 147 samples, MTB detected very low as many as 4 samples, MTB detected low as many as 5 samples, MTB detected medium as many as 5 samples, and the results of examination between microscopes as many as 19 samples from 171 samples (suspected pulmonary TB) and the results of gene Xpert examination as many as 24 samples from 171 samples (suspected pulmonary TB).*

**Keywords---***mycobacterium, patient, pulmonary TB suspect, tuberculosis, Xpert gene microscope.*

## Introduction

In 2017 the World Health Organization (WHO) estimated the incidence of TB at 842,000 or 319 per 100,000 population and deaths due to TB estimated at 107,000 or 40 per 100,000 population in the World (WHO, 2017). Based on that reports or data from the World Health Organization WHO (2018) Asian countries whose TB cases are in TB BURDEN Country are India, China, Philippines Bangladesh, Thailand, and Myanmar. According to data from the Ministry of Health of the Republic of Timor Leste, on this occasion from the disease control program or National

Tuberclose Program TB or National Tuberculosis Program (WHO, 2023). National Tubercolece Program in 2017 the total number of cases was 3620 cases notified from the register book and in 2018 the total number, (50) of cases notified was 3906 cases. Tuberculosis (TB) is an infectious infectious disease caused by Mycobacterium Tuberculosis. This disease is easily stretched and not treated thus can cause complications that lead to death (Moon, 2014; Rajasekaran et al., 2018). Countries with the first cases in the world are India with a presentation of 23%, Indonesia, China ranks second each with a 10% presentation and as tuberculosis sufferers in the world (Directorate General of Prevention Disease Control, 2022; Susilawati & Larasati, 2019; WHO, 2022). Tuberculosis in Timor-Leste is still a complex problem for Timor-Leste. Government and society in general, this is due to the incidence of cases Tuberculosis remained the same as in 2002 until 2016 was the same case at 498 cases per 1000 (498/1000) cases (WHO, 2023).

## Material and Method

### *Research Design*

Research design is experimental research, with *Cross Sectional study approach*. This study aims to measure independent variables and vairabel depedent which can be performed simultaneously at one time (Jacobsen, 2020). This design was chosen with the aim of describing "Mycobacterium detection". Tuberculosis with Microscopic and Gene Xpert examination in Suspect patients Pulmonary Tuberculosis (Sharma et al., 2020; Mustafa et al., 2022).

### *Population and Sample*

The population in this study has two parts Target population of all patients visiting health care facilities in Opportunity Health Service Center in Dili City Affordable population of suspected pulmonary TB patients visiting the center health with certain symptoms such as coughing more than 2 weeks, weight loss sometimes feel fever at night and sometimes cough up mixed with blood. The population in the study was 300 people registered in the TB register from community Health Centre in Dili, Timor-Leste. The sample in this study was 171 respondents, samples for examination using Gene Xpert machine will be referred to INSPTL Laboratory (Natarajan et al., 2020; Frieden & Driver, 2003).

### *Inclusion Criteria*

Patients who are suspected with Pulmonary TB, cough for more than 2 weeks, fever at night, cough mixed with blood, weight loss, decreased appetite, patients registered in the TB laboratory register book, casein sputum or specimen referred to the national laboratory to perform the Gene Xpert test stated as a sample in this study.

### *Exclusion Criteria*

Patients who are not suspected of pulmonary TB, patients who are not registered in the laboratory register book, patients whose Sputum or specimen is not referred to the National laboratory.

## Results dan Discussion

### *Statistical Analysis Results*

Distribution of data based on gender in Dili Municipality is as follows.

Tabel 1  
Distribution of data based on gender in Dili Municipality

No	Gender	Frequency	Percentage (%)
1	Man	82	48
2	Female	89	52
3	Total	171	100

Based on the data in the table above, it shows that this research is 89 (52%) women, 82 (48%) men and a total of 171 (100%) thus it can be concluded that the most are women (52%).

Tabel 2  
Data distribution based on age in Dili Municipality in 2022

No	Age	Frequency	Percentage (%)
1	15 – 25	51	30
2	26 – 35	33	19,3
3	36 – 45	28	16,4
4	46 – 65	33	19,3
5	66+	26	15
	Total	171	100

The table 2 above shows that respondents aged between 15 – 25 are 51 people (30%), 26 – 35 years 33 (19.3%), 36 – 45 years 28 people (16.4%), 46 – 65 33 19.3%), >66 26 people (15%) thus it can be concluded that respondents aged 25-35 (30%) are more involved in this research.

Table 3  
Distribution data based on education level in research responden in Dili Municipality

No	Education Level	Frequency	Percentage (%)
1	Letterless	35	20
2	Elementary School	30	18
3	Junior High School	20	12
4	High School	26	15
5	Undergraduated	60	35
6	Total	171	100

The table 3 above shows that respondents who were less educated 35 (20%), elementary school 30 (18%), junior high school 20 (12%), high school 26 (15%), and undergraduated 60 (35%), and a total of all 171 (100%).

Tabel 4  
Distribution data based on number of samples in Dili Municipality

No	Community Health Center	Frequency	Percentage (%)
1	Comoro	75	44
2	Becora	48	28
3	Verah Cruz	48	28
4	Total	171	100

The table 4 above shows that the total sample was 171 (100%), each community health center in Dili received a sample that was not the same in sampling, the most samples were in the Comoro community health center with a total sample of 75 (44%), the other two community health centers were the same sample 48 (28%).

Table 5  
Data Distribution based on the result of Microscope ezamination in Dili Municipality

Ezamination result	Frequency	Percentage (%)
Negative	152	88.9
Valid		
Scanty	6	3.5
Posetive 1+	10	5.8
Posetive 2+	1	0.6
Posetive 3+	2	1.2

Total	171	100
-------	-----	-----

Based on the table 5 above, it shows that the results of microscopy examination with negative results of 152 (88.9%), scanty 6 (3.5%), positive 1 + 10 (5.8%), positive 2 + 1 (0.6%), and positive results 3 + 2 (1.2%).

Table 6  
Data distribution based on Gene Expert Machine examination result in Dili Municipality

Result Examination		Frequency	Percentage (%)
Valid	Not Detected	147	86.0
	Detected Very Low	4	2.3
	Detected Low	10	5.8
	Detected Medium	5	2.9
	Detected High	5	2.9
Total		171	100.0

The table 6 above shows that the test results using the Gene Xpert machine are not Detected 147 (86%), MTB detected Very Low 4 (2.3%), MTB detected Low 10 (5.8%), MTB Detected Medium 5 (2.9%), MTB Detected High 5 (2.9%).

Tabel 7  
Data distribution based on positive result from Microscope in Dili Municipality

No	Microscope examination results	Frequency	Percentage (%)
1	Positive	19	11
2	Negative	152	89
3	Total	171	100%

Table 7 Results of sputum examination using a positive microscope of acid-resistant bacilli 19 (11%), Negative 152 (89%). It was concluded that the test result was negative (89%).

Tabel 8  
Data distribution based on detected and no detected result using Gene Xpert Machine in suspect TB patient in Dili Municipality

No	Examination results Gene Xpert	Frequency	Percentage (%)
1	Detected	24	14
2	Non-Detected	147	86
3	Total	171	100%

Table 8 above shows that mycobacterium tuberculosis examination using Gene Xpert machine, detected mycobacterium Tuberculosis 24 (14%), Not detected 147 (86%), from total sputum samples 171 (100%) ([Marciniuk et al., 1999](#); [McAdams et al., 1995](#)).

## Discussion

Based on the results of research using 2 different methods, namely the Microscope and Gene Xpert tools showed that the positive results of the Microscope 19 (11%) while the Gene Xpert machine detected 24 (14%) results showed that the Gene Xpert machine was better than the Microscope [Khan et al. \(2018\)](#); [Najjingo et al. \(2019\)](#), where the results of the microscope examination using the Zeel Nelsen method with the results of 152 negative samples while using the Gene Xpert 147 machine, negative samples with a difference of 3% thus it can be concluded that the Gene Xpert machine as the Gold standard in diagnosing suspected tuberculosis disease, in other words the Gene Xpert machine is better than the microscope, this study is the same as that conducted [Meawed & Shaker \(2016\)](#), although only a slight difference of 3% ([Dailloux et al., 1999](#); [Koh et al., 2012](#)).

Based on the results of research conducted on the 25th of April to the 9<sup>th</sup> June 2022 Dili Municipality by obtaining results that from 171 samples that have been perform examinations using the Microscope and Gene Xpert examination methods with the following results microscope examination using the Zeel Nelsen method from 171 samples (suspected Pulmonary TB) using the IUATLD (International Union Against Tuberculosis and Lung Disease) WHO (2023) calculation scale at examination up to 100 views with negative as many as 152 samples are equivalent to (89%) (Aaron et al., 2004; Millar & Horne, 1979). While using the Gene Xpert Machine method with Catrige with MTB results not Detected as many as 147 samples equivalent to (86%). Based on the results of TB suspect examination lungs using a Gene Xpert machine and a microscope tool there are differences results, where the Xpert gene machine showed the results of 147 negative samples from 171 samples of suspected pulmonary TB, while using a microscope showed results of 152 negative samples from 171 suspected samples of Pulmonary TB. Based on statistict analysis tests shows that the difference in results between the microscope and Gene Xpert is different although not significant between the results of examination using a microscope and a gene Xpert machine in suspected Pulmonary Tuberculosis patients, this study is the same as that conducted by Arora & Dhanashree (2020); Kabir et al. (2021), this difference is the number of bacteria from each method used. His research showed that the detection of Mycobacterium Tuberculosis with the Xpert Gene method is at least 131 bacteria / ml of sputum while the method microscope detects Mycobacterium Tuberculosis with a total of 5000 bacteria / ml of sputum but the number that can be detected is only a few bacteria. Xpert gene machines can detect Mycobacterium Tuberculosis in small numbers compared to Microscope that can only be read if the number of bacterial cells is large (Huang et al., 2023; Chakravorty et al., 2017).

## Conclusion

Detection of mycobacterium tuberculosis using microscope and Xpert gene in patients Pulmonary Tuberculosis Suspect in Dili district can be concluded that Mycobakterium examination Tuberculosis using a microscope from 171 samples with bacilli examination results acid resistance as much as 19 positive 11 (11%). Mycobacterium Tuberculosis examination using Gene Xpert machine from 171 samples 24 Detected (14%).

## Acknowledgements

This work was supported by a Universidad da PAZ East Timor-Leste Promotor and Co-Promotor. All Community Health Centre in Dili Municipality.

## Declaration of Interest

The authors declare that they have no conflict of interest in this research.

## References

- Aaron, L., Saadoun, D., Calatroni, I., Launay, O., Memain, N., Vincent, V., ... & Lortholary, O. (2004). Tuberculosis in HIV-infected patients: a comprehensive review. *Clinical microbiology and infection*, 10(5), 388-398. <https://doi.org/10.1111/j.1469-0691.2004.00758.x>
- Arora, D., & Dhanashree, B. (2020). Utility of smear microscopy and GeneXpert for the detection of Mycobacterium tuberculosis in clinical samples. *Gems*, 10(2), 81.
- Chakravorty, S., Simmons, A. M., Rowneki, M., Parmar, H., Cao, Y., Ryan, J., ... & Alland, D. (2017). The new Xpert MTB/RIF Ultra: improving detection of Mycobacterium tuberculosis and resistance to rifampin in an assay suitable for point-of-care testing. *MBio*, 8(4), 10-1128.
- Dailloux, M., Laurain, C., Weber, M., & Hartemann, P. H. (1999). Water and nontuberculous mycobacteria. *Water Research*, 33(10), 2219-2228. [https://doi.org/10.1016/S0043-1354\(98\)00466-7](https://doi.org/10.1016/S0043-1354(98)00466-7)
- Directorate General of Prevention Disease Control. (2022). Tuberculosis Control in Indonesia 2022. Jakarta: Ministry of Health of the Republic of Indonesia.
- Frieden, T. R., & Driver, C. R. (2003). Tuberculosis control: past 10 years and future progress. *Tuberculosis*, 83(1-3), 82-85. [https://doi.org/10.1016/S1472-9792\(02\)00060-4](https://doi.org/10.1016/S1472-9792(02)00060-4)
- Huang, W., Lee, M. K. T., Sin, A. T. K., Nazari, R. S., Chua, S. Y., & Sng, L. H. (2023). Evaluation of Xpert MTB/RIF ultra assay for detection of Mycobacterium tuberculosis and rifampicin resistance. *Pathology*.
- Jacobsen, K. H. (2020). *Introduction to health research methods: A practical guide*. Jones & Bartlett Publishers.

- Kabir, S., Parash, M. T. H., Emran, N. A., Hossain, A. T., & Shimmi, S. C. (2021). Diagnostic challenges and GeneXpert utility in detecting Mycobacterium tuberculosis among suspected cases of Pulmonary tuberculosis. *Plos one*, *16*(5), e0251858.
- Khan, A. S., Ali, S., Khan, M. T., Ahmed, S., Khattak, Y., Irfan, M., & Sajjad, W. (2018). Comparison of GeneXpert MTB/RIF assay and LED-FM microscopy for the diagnosis of extra pulmonary tuberculosis in Khyber Pakhtunkhwa, Pakistan. *brazilian journal of microbiology*, *49*, 909-913.
- Koh, W. J., Jeong, B. H., Jeon, K., Lee, N. Y., Lee, K. S., Woo, S. Y., ... & Kwon, O. J. (2012). Clinical significance of the differentiation between Mycobacterium avium and Mycobacterium intracellulare in M avium complex lung disease. *Chest*, *142*(6), 1482-1488. <https://doi.org/10.1378/chest.12-0494>
- Marciniuk, D. D., Mc Nab, B. D., Martin, W. T., & Hoepfner, V. H. (1999). Detection of pulmonary tuberculosis in patients with a normal chest radiograph. *Chest*, *115*(2), 445-452. <https://doi.org/10.1378/chest.115.2.445>
- McAdams, H. P., Erasmus, J., & Winter, J. A. (1995). Radiologic manifestations of pulmonary tuberculosis. *Radiologic Clinics of North America*, *33*(4), 655-678. [https://doi.org/10.1016/S0033-8389\(22\)00611-X](https://doi.org/10.1016/S0033-8389(22)00611-X)
- Meawed, T. E., & Shaker, A. (2016). Assessment of diagnostic accuracy of Gene Xpert MTB/RIF in diagnosis of suspected retreatment pulmonary tuberculosis patients. *Egyptian Journal of Chest Diseases and Tuberculosis*, *65*(3), 637-641.
- Millar, J. W., & Horne, N. W. (1979). Tuberculosis in immunosuppressed patients. *The Lancet*, *313*(8127), 1176-1178. [https://doi.org/10.1016/S0140-6736\(79\)91852-X](https://doi.org/10.1016/S0140-6736(79)91852-X)
- Moon, M. S. (2014). Tuberculosis of spine: current views in diagnosis and management. *Asian spine journal*, *8*(1), 97.
- Mustafa, H., Shah, N. N., Shah Nawaz, M., & Yousuf, M. (2022). Role of Gene Xpert in smear negative pulmonary tuberculosis. *Indian Journal of Tuberculosis*, *69*(4), 552-557. <https://doi.org/10.1016/j.ijtb.2021.08.035>
- Najjingo, I., Muttamba, W., Kirenga, B. J., Nalunjogi, J., Bakesiima, R., Olweny, F., ... & Ssengooba, W. (2019). Comparison of GeneXpert cycle threshold values with smear microscopy and culture as a measure of mycobacterial burden in five regional referral hospitals of Uganda-A cross-sectional study. *PLoS One*, *14*(5), e0216901.
- Natarajan, A., Beena, P. M., Devnikar, A. V., & Mali, S. (2020). A systemic review on tuberculosis. *Indian Journal of Tuberculosis*, *67*(3), 295-311. <https://doi.org/10.1016/j.ijtb.2020.02.005>
- Rajasekaran, S., Soundararajan, D. C. R., Shetty, A. P., & Kanna, R. M. (2018). Spinal tuberculosis: current concepts. *Global Spine Journal*, *8*(4\_suppl), 96S-108S.
- Sharma, J. B., Dharmendra, S., Jain, S., Sharma, S. K., Singh, U. B., Soneja, M., ... & Vanamail, P. (2020). Evaluation of Gene Xpert as compared to conventional methods in diagnosis of Female Genital Tuberculosis. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *255*, 247-252. <https://doi.org/10.1016/j.ejogrb.2020.09.046>
- Susilawati, T. N., & Larasati, R. (2019). A recent update of the diagnostic methods for tuberculosis and their applicability in Indonesia: a narrative review. *Medical Journal of Indonesia*, *28*(3), 284-91.
- WHO. (2017). Global Tuberculosis Report. Geneva: World Health Organization: WHO Media Centre.
- WHO. (2018). Global Tuberculosis Report. Geneva: World Health Organization: WHO Media Centre.
- WHO. (2022). TB Case. Global Tuberculosis Report 2022.
- WHO. (2023). Democratic Republic of Timor-Leste Ministry of Health - Comprehensive TB Guidelines for National Tuberculosis Program, V Edition. Timor leste: WHO Timor Leste.