Jurnal Kesehatan Manarang

Jurnal Kesehatan Manarang, Volume 9, Number 3, December 2023, pp. 215 – 221
ISSN 2528-5602 (Online), ISSN 2443-3861 (Print)
doi: https://doi.org/10.33490/jkm.v9i3.848

COMPARISON OF TUBERCULOSIS EXAMINATION USING ZIEHL-NEELSEN METHOD AND MOLECULAR RAPID TEST

Aan Yulianingsih Anwar[1]✉, Marini Tri Putri Handayani[2], Erpi Nurdin[3], Irma B.Hi.Lewa[4], Artati[5]

[1,2,3,4]Departmen of Medical Laboratory Technology, Health Polytechnic of Ternate
[5] Departmen of Medical Laboratory Technology, Health Polytechnic of Makassar

ARTICLE INFO

Article history

Submitted : 2023-01-20
Revised : 2023-12-13
Accepted : 2023-12-19

Keywords:

Moleculer Rapid Test;
Tuberculosis;
Ziehl-Neelsen;

ABSTRACT

Tuberculosis is an infectious disease of the respiratory tract caused by infection with the bacteria Mycobacterium tuberculosis. Fast and accurate diagnostic methods are very important for diagnosing and controlling pulmonary TB disease in Indonesia. TB examination using the Zn method is very commonly used even though it has lower sensitivity than TCM. The aim of this study was to compare the results of TB examination using BTA staining with the Molecular Rapid Test. This type of research uses analytical observational methods with a cross-sectional approach. The sample was 40 respondents taken using purposive sampling technique. The results of tuberculosis examination using the Ziehl-Neelsen (ZN) BTA staining method and the Moluker rapid test tool (TCM) method showed that the microscopic results of the Ziehl-Neelsen method of BTA smear staining showed negative results in 31 people (75%), 1 respondent (2 .5%) with scanty results and 9 respondents (22.5%) with 1+ results. The results of the TB BTA examination using the TCM method resulted in MTB Not Detected with a total of 30 people (75%), MTB Detected Medium obtained a result of 6 people (15%), MTB Detected Low obtained a result with a total of 3 people (7.5%), MTB Detected Very Low results were obtained with the number of 1 person (2.5%) and in the SPSS results using the t-test the results obtained were p value <0.05 (0.012 <0.05) which means there is a comparison of the BTA staining tuberculosis examination using the Ziehl-Ziehl method. neelsen and Molecular Rapid Tests. Based on the research results, it was found that there was a significant difference between the Ziehl-Neelsen BTA staining tuberculosis examination method and the Molecular Rapid Test Tool with a p value <0.05 (0.012 <0.05)

Kata Kunci:

Tes cepat molekuler;
Tuberkulosis;
Ziehl-Neelsen;

ABSTRAK

Tuberculosis merupakan penyakit infeksi pada saluran pernapasan yang disebabkan oleh infeksi bakteri Mycobacterium tuberculosis. Metode diagnostik yang cepat dan akurat sangat penting untuk penegakan diagnosis dan pengendalian penyakit TB paru di Indonesia. Pemeriksaan TB dengan menggunakan metode Zn sangat umum digunakan walaupun memiliki sensitifitas yang rendah dibandingkan TCM. Tujuan dari penelitian ini untuk mengetahui perbandingan hasil pemeriksaan TBC menggunakan perwarnaan BTA dengan alat Tes Cepat Molekuler. Jenis penelitian menggunakan metode observasional analitik dengan pendekatan cross-sectional. Sampel sebanyak 40 responden yang diambil dengan Teknik puposive sampling. Hasil pemeriksaan tuberculosis menggunakan pewarnaan BTA metode ziehl-neelsen (ZN) dan metode Alat tes cepat moluker (TCM) menunjukkan bahwa hasil mikroskopik pemeriksaan TBC BTA metode ziehl-neelsen didapat hasil negatif dengan jumlah 31 orang (75%.), 1 responden (2,5%) dengan hasil scanty dan 9 responden (22,5%) dengan hasil 1+. Hasil pemeriksaan TBC BTA Metode TCM didapat hasil MTB Not Detected denga jumlah 30 orang (75%), MTB Detected Medium didapat hasil 6 orang (15%), MTB Detected Low didapat hasil dengan jumlah 3 orang (7,5%), MTB Detected Very Low didapat hasil dengan jumlah 1 orang (2,5%) dan pada hasil SPSS dengan menggunakan uji-t diperoleh hasil nilai p<0,05 (0,012<0,05) yang berarti ada perbandingan pemeriksaan tuberculosis pewarnaan BTA metode ziehl-neelsen dan Tes Cepat Molekuler. Berdasarkan hasil penelitian didapatkan ada perbedaan yang bermakna antara pemeriksaan tuberkulosis pewarnaan BTA metode Ziehl-Neelsen dan Alat Tes Cepat Molekuler dengan nilai p<0,05 (0,012<0,05)

✉ ***Corresponding Author:***

Aan Yulianingsih Anwar
Telp. 081340032281
Email: aanyulianingsihanwar@gmail.com

INTRODUCTION

Tuberculosis is an infectious disease affecting the respiratory system caused by the bacterium Mycobacterium tuberculosis. This bacterium commonly attacks the lungs and, in some cases, other organs such as lymph nodes, skin, intestines or the digestive tract, meninges, and so forth (Aini et al., 2017). Mycobacterium tuberculosis, also known as acid-fast bacilli (AFB), is a robust intracellular pathogenic bacterium causing tuberculosis infection, which takes a long time to heal. This disease remains a leading cause of death worldwide (Andayani & Astuti, 2017).

According to the World Health Organization (WHO), tuberculosis is a global health problem following HIV, ranking among the top 10 causes of death worldwide. Geographically, the majority of TB cases in 2018 were in the Southeast Asia region (44%), Africa (24%), and the Western Pacific (18%), with smaller percentages in the Eastern Mediterranean (8%) and the Americas (3%) (World Health Organization, 2019).

Indonesia is among the top three countries with the highest number of TB cases. In Indonesia, TB cases increased from 331,703 in 2015 to 563,879 in 2018 (+70%), including an increase of 121,707 (+28%) between 2017 and 2018. In 2018, the number of TB cases by gender in Indonesia was 294,757 cases in males and 217,116 cases in females. Thus, in 2018, TB cases in Indonesia totaled 511,873 cases (Kementerian Kesehatan Republik Indonesia, 2019).

The Ternate City Health Office reported that in 2019, there were 527 TB cases in Ternate City (prevalence of 226/100,000 population), with a breakdown by gender of 288 males and 239 females. The number of deaths from TB cases was found to be 23 people (10/100,000 population). Reported TB cases are still relatively high in Ternate City, requiring serious prevention efforts to curb the increase in cases (Dinas Kesehatan Kota Ternate, 2019).

Swift and accurate diagnosis of tuberculosis is crucial to address the limitations of TB detection methods, resulting in delays in tuberculosis treatment. Rapid and accurate diagnostic methods are essential for the confirmation and control of pulmonary TB in Indonesia. Minister of Health Regulation No. 67 of 2016 on Tuberculosis Control recommends two microscopic examination methods with Ziehl-Neelsen (ZN) staining and molecular rapid tests with GeneXpert MTB/RIF. These examinations are carried out according to the availability of healthcare facility infrastructure.

Microscopic examination with Ziehl-Neelsen staining to observe acid-fast bacilli (AFB) has less satisfactory sensitivity compared to GeneXpert MTB/RIF. Research by Kashif Munir et al. (2015) indicates that the positivity value, sensitivity, and specificity of ZN staining of lung specimens for diagnosing pulmonary TB are 67.5%, 77.7%, and 91.4%, respectively, while the positivity and specificity of GeneXpert MTB/RIF in pulmonary TB with negative AFB are 88.33% and 95.46%, respectively.

The lack of internal quality control (IQC) measurements, uneven, thin, and improperly sized smears are related to false-negative results in ZN staining. Factors associated with false-positive results include the absence of IQC measurements and poor staining procedures (Mekonen et al., 2018). Some limitations of GeneXpert MTB/RIF include the inability to monitor TB treatment success, differentiate between viable and non-viable MTB, require a cooling system to maintain a temperature between 15-25°C, and require electricity throughout the reaction, making it unsuitable for locations with limited electricity (Khairunisa, 2017). Based on the background described, the objective of this study is to analyze the comparison of Tuberculosis examinations using ZN staining and molecular rapid tests equipment.

METHODS

Study Design

This study employed an analytical observational design with a *cross-sectional* approach.

Location and Study Period

The research was conducted at Kalumata Community Health Center, Ternate City. The study took place from May 1 to June 1, 2022.

Population and Sample

The population consisted of 90 respondents who were suspected TB patients aged ≥17 years and whose sputum examination revealed *Mycobacterium tuberculosis* bacteria at Kalumata Community Health Center. The sample for this study included 40 respondents selected through purposive sampling technique.

Data Collection

The instruments used in this study were microscope slides, wooden sticks, forceps, staining racks, sputum sample containers, microscope, cartridge/module, GeneXpert molecular rapid tests, Carbol fuchsin 1%, Acid alcohol 3%, Methylene blue 0.1%.

For sputum sampling, sterile wide-mouthed containers (single-use) were used. The containers were labeled with the patient's TB identification, and the examination steps were as follows:

1. Preparation of Slides
   a. Assign an identification number to the specimen.
   b. Create an oval-shaped sputum smear measuring 2x3 cm.
   c. Flatten the sputum using a pointed stick in a spiral motion before it dries.
   d. Dry the specimen at room temperature.
   e. Hold the microscope slide with forceps, with the specimen surface facing upwards.
   f. Pass it over the blue flame 2-3 times for 1-2 seconds.
2. Ziehl-Neelsen Staining
   a. Flood the entire specimen surface with 3% Carbol Fuchsin.
   b. Heat until vapor is emitted, then cool for 5 minutes.
   c. Rinse the specimen with 0.3% acid alcohol until there is no residual red color, then rinse with flowing water.
   d. Flood the entire specimen surface with 3% Methylene Blue.
   e. Rinse with water.
3. Microscopic Reading and Reporting with IUATLD Scale
   a. Locate the LP at 10x10 magnification.
   b. Drop immersion oil onto the specimen, observe at 10x100 magnification.
   c. Read along the centerline, 100 LP.
4. Standard Operating Procedure for Xpert MTB/RIF
   a. Open the Sample Reagent (SR) seal and the tube cap containing the sputum sample.
   b. Pour SR into the sputum tube with SR volume twice the sputum volume. Close the sputum tube again.
   c. Shake 10-20 times, then incubate for 10 minutes. Shake vigorously again, then incubate for 5 minutes. If the sputum is still thick and clotted, extend the incubation time by 5-10 minutes.
   d. Prepare the Xpert MTB/RIF cartridge, label it on the right or left side using a marker or barcode sticker.
   e. Open the top of the cartridge.
   f. Transfer the processed sputum using the provided pipette, filling the pipette beyond the 2 mL mark.
   g. Slowly insert the pipette into the sample chamber on the cartridge, then slowly release the sputum. Avoid air bubble formation.
   h. Close the cartridge tightly. Process immediately using the GeneXpert machine. Wait for the results.

Data Processing and Analysis

The data analysis in this research is observational and analytical, employing a cross-sectional approach to examine the characteristics of respondents (gender and age), the distribution of TB examination results using the Ziehl-Neelsen (ZN) method, and the distribution of TB examination results using the Molecular Rapid Test. The data obtained are analyzed using paired t-tests to compare the tuberculosis (TB) examinations using ZN staining and the Molecular Rapid Test.

RESULTS

The characteristics of TB patients under study include gender and age. The results of each frequency distribution of TB patients can be seen in Table 1 below.

Table 1. Frequency Distribution of TB Patients

No	Criteria	Frequency (N=40)	Percentage (%)
1	**Respondent's Age**		
	17-25	5	12,5
	26-35	7	17,5
	36-45	8	20
	46-55	13	32,5
	56-65	7	17,5
2	**Gender**		
	Male	24	60
	Female	16	40

Table 1 above shows that out of 40 respondents, 13 respondents (32.5%) were in the age group of 46-55 years, and the male gender was represented by 24 respondents (60%).

Table 2. Distribution of TB Examination Results Ziehl-Neelsen Method

No	Microscopic Results	F	P (%)
1.	Negative	30	75
2.	Scanty	1	2,5
3.	1+	9	22,5
4.	2+	-	0
5.	3+	-	0
	Total	**40**	**100**

Table 2 shows that the microscopic results of the Ziehl Neelsen Method of acid-fast bacteria TB examination were negative for 30 people (75%) and 9 respondents (22.5%) with results of 1+.

Table 3. Distribution of TB Examination Results by Molecular Rapid Test Method

No	Results	F	P (%)
1	MTB Not Detected	30	75
2	MTB Detected High	-	0
3	MTB Detected Medium	6	15
4	MTB Detected Low	3	7,5
5	MTB Detected Very Low	1	2,5
6	MTB Detected High	-	0
7	MTB Detected Very High	-	0
	Total	**40**	**100**

Table 3 shows that the results of the Molecular Rapid Test acid-fast bacteria TBC examination obtained MTB Not Detected results with a total of 30 people (75%) and MTB Detected Very Low results obtained with a total of 1 person (2.5%).

Tabel 4. Comparison of Tuberculosis Examination Results using Molecular Rapid Test and Ziehl-Neelsen Method

Ziehl-Nelseen	**Molecular Rapid Test**					**Nilai p Value**
	MTB Not Detected	MTB Detected very low	MTB Detected Low	MTB Detected Medium	MTB Detected High	
Negative	30					0,012
Scanty	1					
1+		1	3	6		
2+						
3+						

Table 4 above indicates that the comparison results of the Ziehl-Neelsen Method show negative results in 30 respondents, scanty results in 1 respondent, and positive 1+ results in 9 respondents. The examination results using the Molecular Rapid Test Method identified MTB Not Detected in 30 respondents, MTB Detected Very Low in 1 respondent, and MTB Detected Medium in 6 respondents.

Based on the cross-tabulation test in Table 4 using the paired T-test, the obtained p-value is 0.012 ($p<0.05$), indicating that there is a statistically significant difference in the TB examination results between the Ziehl-Neelsen and Molecular Rapid Test methods.

DISCUSSION

Table 1 indicates that out of 40 respondents, the majority suffering from Tuberculosis are in the age group of 46-55 years, accounting for 32.5%. As a person's age increases, the risk of TB also increases. Older individuals tend to have a weakened immune system, making them more susceptible to diseases. Participants in the 55 years age group have a 1.73 times higher risk of TB compared to those in younger age groups (Pangaribuan et al., 2020).

Table 1 also reveals that males are more prone to TB compared to females. This can be attributed to the fact that males engage in more outdoor activities, increasing their chances of contracting TB. Additionally, habits such as alcohol consumption and smoking among males can weaken their immune systems, making them more susceptible to TB (Agustian et al., 2022). This aligns with a study by Sunarmi (2022), which found a significant relationship between gender and the incidence of tuberculosis (p-value = 0.093<0.10) (Sunarmi & Kurniawaty, 2022).

Fast and accurate diagnostic methods are crucial for the diagnosis and control of pulmonary TB in Indonesia. The Ministry of Health of the Republic of Indonesia recommends two types of microscopic examinations with Ziehl-Neelsen (ZN) staining and molecular rapid tests with GeneXpert MTB/RIF, based on the availability of healthcare facilities (Ministry of Health of the Republic of Indonesia, 2016).

The ZN staining principle relies on the ability of MTB to retain carbol fuchsin dye after decolorization with acid-alcohol (Suryawati et al., 2019). The working principle of GeneXpert MTB/RIF involves real-time polymerase chain reaction (PCR), amplifying 81 base pairs on the rpoB gene of MTB and using five probes (A-E) (Kesuma et al., 2020).

One of the priorities in TB control is the early detection of TB cases. Microscopic examination of acid-fast bacilli (AFB) is a widely used diagnostic method. While AFB microscopy has the advantage of being cost-effective, it has limitations. Sputum must contain a minimum of 5000 bacteria/ml for a positive result. Another drawback is the difficulty clinicians face in diagnosing TB in patients with scanty microscopic results (Amala et al., 2020; Kurniawan et al., 2016).

Table 3 shows the results of TB examination using the molecular rapid tests method. The results include 30 respondents (75%) identified as MTB Not Detected, 6 respondents (15%) as MTB Detected Medium, 3 respondents (7.5%) as MTB Detected Low, and 1 respondent (2.5%) as MTB Detected Very Low. The Molecular Rapid Test can be completed within two hours or even less. Large-scale trials have assessed the accuracy of Xpert MTB/RIF, with a sensitivity of up to 98% for detecting both positive and

negative TB bacteria. However, there is a 50% sensitivity in testing for TB in HIV patients (Relasiskawati, 2016).

In Table 4, the obtained p-value is 0.012, indicating a significant difference in TB examination results between the ZN and TCM methods. The difference is observed in the 1+ result in the ZN method, while in the TCM method, it is identified in MTB Detected Very Low, MTB Detected Low, and MTB Detected Medium.

The scale based on IUALTD (International Union Against Lung Disease) indicates a negative result in microscopic examination if no AFB is found in 100 fields of view. The difference in microscopic and TCM results occurs because microscopic examination in sputum must contain a minimum of 5000 bacteria/ml for accurate results in AFB. The abundance of mucus will increase the sample volume, reducing the likelihood of obtaining a sample containing M. tuberculosis. On the other hand, TCM examination with molecular detection can qualitatively detect DNA from the MTB complex but cannot be used as a follow-up examination (monitoring) in patients undergoing treatment (Nurdiani et al., 2022; Kementerian Kesehatan Republik Indonesia, 2017).

The differences in GeneXpert examination results compared to microscopic examination scores are due to the different methods used in the two examinations. GeneXpert examination uses an automated system that integrates specimen purification, nucleic acid amplification, and target sequence detection. The GeneXpert cartridge has a Sample Processing Control (SPC) and Probe Check Control (PCC). SPC functions as a process control adequate for the target bacteria and to monitor the presence of inhibitors in the PCR reaction. Meanwhile, PCC ensures the rehydration process of reagents, filling the PCR tube in the cartridge, probe integrity, and dye stability (Kementerian Kesehatan Republik Indonesia, 2017).

CONCLUSION AND RECOMMENDATIONS

The paired T-test analysis results indicate a significant difference in TB examination results between the Ziehl-Neelsen and Molecular Rapid Test methods (p=0.012). It is recommended for healthcare facilities to use methods with good sensitivity and specificity to obtain accurate results.

DAFTAR PUSTAKA

Agustian, M. D., Masria, S., & Ismawati. (2022). Hubungan Usia , Jenis Kelamin dan Tingkat Pendidikan dengan Kejadian TB Paru di Wilayah Kerja Puskesmas Cibadak Kabupaten Sukabumi. *Bandung Conference Series: Medical Science*, *2*(1), 1120–1125. https://doi.org/10.29313/bcsms.v2i1.2256

Aini, N., Ramadiani, R., & Hatta, H. R. (2017). Sistem Pakar Pendiagnosa Penyakit Tuberkulosis. *Informatika Mulawarman : Jurnal Ilmiah Ilmu Komputer*, *12*(1), 56. https://doi.org/10.30872/jim.v12i1.224

Amala, S. E., Hanson, A., & Wokem, G. N. (2020). Co-Infection with Mycobacterium tuberculosis in Tuberculosis Patients and Antifungal Susceptibility of the Isolates. *Journal of Tuberculosis Research*, *08*(02), 53–65. https://doi.org/10.4236/jtr.2020.82006

Andayani, S., & Astuti, Y. (2017). Prediksi Kejadian Penyakit Tuberkulosis Paru Berdasarkan Usia Di Kabupaten Ponorogo Tahun 2016-2020. *Indonesian Journal for Health Sciences*, *1*(2), 29. https://doi.org/10.24269/ijhs.v1i2.482

Dinas Kesehatan Kota Ternate. (2019). *Laporan Triwulan Penemuan Pasien Tuberculosis*.

Kashif Munir, M., Rehman, S., Aasim, M., Iqbal, R., & Saeed, S. (2015). Comparison of Ziehl Neelsen Microscopy with GeneXpert for Detection of MycobacteriumTuberculosis. *IOSR Journal of Dental and Medical Sciences*, *14*(11), 2279–2861. https://doi.org/10.9790/0853-1411105660

Kementerian Kesehatan Republik Indonesia. (2016). *Peraturan Menteri Kesehatan Indonesia No. 67 Tahun (2016) tentang Penanggulangan Tuberculosis*. https://peraturan.bpk.go.id/Details/114486/permenkes-no-67-tahun-2016

Kementerian Kesehatan Republik Indonesia. (2017). *Data dan informasi Kesehatan Profil Kesehatan Indonesia(2016)*. https://www.bps.go.id/id/publication/2017/12/19/75a48175e339189155cc348e/profil-statistik-kesehatan-2016.html

Kementerian Kesehatan Republik Indonesia. (2019). *Indonesian health profile data and information(2018)*. https://www.kemkes.go.id/app_asset/file_content_download/profil-kesehatan-indonesia-2018.pdf?utm_medium=email&utm_source=transaction

Kementerian Kesehatan RI. (2017). Petunjuk Teknis Pemeriksaan TB dengan TCM. *Kementerian Kesehatan RI*, 1–170. www.tbindonesia.or.id

Kesuma, S., Abdullah, T., & Laboratorium Medis Poltekkes Kemenkes Kalimantan Timur Samarinda Kalimantan Timur Indonesia, T. (2020). Uji Diagnostik Gene Xpert Mtb/Rif Pada Pemeriksaan Mycobacterium Tuberculosis Di RSUD R. Syamsudin Sh Kota Sukabumi. *Husada Mahakam : Jurnal Kesehatan*, *10*(2), 94–101. https://doi.org/10.35963/hmjk.v10i2.214

Khairunisa, S. (2017). An activist's guide to Tuberculosis Diagnostic Tools. *Treatment Action Group*, *February*. https://www.treatmentactiongroup.org/wp-content/uploads/2017/04/TB-Diagnostics-Guide.pdf

Kurniawan, E., Raveinal, R., Fauzar, F., & Arsyad, Z. (2016). Nilai Diagnostik Metode "Real Time" PCR GeneXpert pada TB Paru BTA Negatif. *Jurnal Kesehatan Andalas*, *5*(3), 730–738. https://doi.org/10.25077/jka.v5i3.609

Mekonen, A., Ayele, Y., Berhan, Y., Woldeyohannes, D., Erku, W., & Sisay, S. (2018). Factors which contributed for low quality sputum smears for the detection of acid fast bacilli (AFB) at selected health centers in Ethiopia: A quality control perspective. *PLoS ONE*, *13*(6), 1–12. https://doi.org/10.1371/journal.pone.0198947

Nurdiani, C. U., Kristianingsih, Y., & Zahrawani, A. Q. (2022). Gambaran Hasil Pemeriksaan BTA dan Genexpert Pada Pasien Suspek Tuberkulosis Di RSUD Budhi Asih Jakarta Timur. kesehatan masyarakat. (Kasus Penyakit Menular d. *Anakes: Jurnal Ilmiah Analis Kesehatan*, *8*(1), 11–19. https://doi.org/10.37012/anakes.v8i1.873

Pangaribuan, L., Kristina, K., Perwitasari, D., Tejayanti, T., & Lolong, D. B. (2020). Faktor-Faktor yang Mempengaruhi Kejadian Tuberkulosis pada Umur 15 Tahun ke Atas di Indonesia. *Buletin Penelitian Sistem Kesehatan*, *23*(1), 10–17. https://doi.org/10.22435/hsr.v23i1.2594

Relasiskawati. (2016). Uji Kesesuaian Hasil Pemeriksaan Mikroskopis Basil Tahan Asam Metode Ziehl-Neelsen Dengan Tes Cepat Molekuler (GeneXpert) Pada Pemeriksaan Tuberculosis Paru Dari Sampel Sputum. (Issue July). http://repo.upertis.ac.id/id/eprint/1552

Sunarmi, S., & Kurniawaty, K. (2022). Hubungan Karakteristik Pasien Tb Paru Dengan Kejadian Tuberkulosis. *Jurnal 'Aisyiyah Medika*, *7*(2), 182–187. https://doi.org/10.36729/jam.v7i2.865

Suryawati, B., Saptawati, L., Putri, A. F., & Aphridasari, J. (2019). Sensitivitas Metode Pemeriksaan Mikroskopis Fluorokrom dan Ziehl-Neelsen untuk Deteksi Mycobacterium tuberculosis pada Sputum. *Smart Medical Journal*, *1*(2), 56. https://doi.org/10.13057/smj.v1i2.28704

Word Health Organization. (2019). *Global Tuberculosis Report*. https://www.who.int/publications/i/item/9789241565714