International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Comparing the Result of Xpert MTB/RIF Assay with iiPCR POCKIT TB in TB Suspect

Ambar Triana[1], Kristanti Herietrenggi[2], Abas Suherli[3]

[1]Study Program of Medical Laboratory Technology Polytechnic Ministry of Health Jakarta III, Dr. Chasbullah Abdulmadjid Hospital, Bekasi City

[2]Study Program of Medical Laboratory Technology Polytechnic Ministry of Health Jakarta III, Dr. Chasbullah Abdulmadjid Hospital, Bekasi City
Email: *kristantiherietrenggi75[at]gmail.com*

[3]Study Program of Medical Laboratory Technology Polytechnic Ministry of Health Jakarta III, Dr. Chasbullah Abdulmadjid Hospital, Bekasi City

Abstract: ***One of the strategies of the Indonesian government for eliminating tuberculosis (TB) is the provision of sensitive and specific diagnostic tools to diagnose Mycobacterium tuberculosis. Besides culture examination as the gold standard, molecular - based methods such as Xpert MTB/RIF and isolated isothermal polymerase chain reaction (iiPCR) exist. The purpose of this study was to compare the result of TB examination between Xpert MTB/RIF assay with iiPCR Pockit TB assay in patients with suspected TB at dr. Chasbullah Abdulmadjid Hospital. The study method is analytical observational with a cross - sectional approach using 47 sputum samples. TheXpert MTB/RIF examination results were compared with the iiPCR Pockit. Data were analyzed by cross - tabulation and Kappa. It showed value using Xpert MTB/RIFassay 38 (80.9%) positive results were obtained, and 37 (78, 7%) samples were detected positive for iiPCR Pockit TB. The comparison of results found that there were 3 (6.4%) samples whose results did not match the two methods. Based on Kappa Cohen's statistical analysis, the kappa value of 0.802 means there is a good agreement, and the standard error value of 0.109 with a significant level of 0.000 means there is no statistically significant difference between the Xpert MTB/RIF and iiPCR Pockit TB to detecting Mycobacterium tuberculosis. Thus, iiPCRPockit TB may serve as an alternative method for initial screening and provide a valuable platform for TB detection.***

Keywords*:* iiPCR Pockit, Tuberculosis, Xpert MTB/RIF

1. Introduction

Tuberculosis (TB) is still a public health problem, even though efforts to control TB have been implemented in many countries. Since the start of 2020, the COVID - 19 pandemic has had enormous health, social and economic impacts, which are likely to continue in 2021 and beyond; even after some of these impacts have been mitigated, there will be medium and long - term consequences, including for the epidemic and control of tuberculosis. Globally, an estimated 10.0 million (range 8.9 - 11.0 million) people fell ill with TB in 2019, declining very slowly in recent years (WHO, 2020).

The economic difficulties that TB directly and indirectly causes create barriers to access to diagnosis and treatment, which can worsen treatment outcomes and increase the risk of transmission of infection in the community. This situation certainly hampers several development goals in the health sector at the global, national, and regional levels (Perpres, 2021). The extra pressure on health services due to the COVID - 19 pandemic, especially in countries with a high burden of TB, combined with the impact on tracking TB sufferers, can slow down treatment and prevention targets (WHO, 2020).

Data from the WHO Global TB Report 2020, geographically, most TB sufferers in 2019 are in Southeast Asia (44%), Africa (25%), and West Pacific (18%) regions, with a smaller percentage in the Eastern Mediterranean (8.2%), America (2.9%) and Europe (2.5%). The eight countries contributing to two - thirds of the global total are India (26%), Indonesia (8.5%), China (8.4%), Philippines (6.0%), Pakistan (5.7%), Nigeria (4.4%), Bangladesh (3.6%) and South Africa (3.6%) (WHO, 2020).

Tuberculosis has caused a complex problem both from a medical and social, economic, and cultural perspective. Based on the WHO Global TB Report 2020, Indonesia is the country with the second highest TB burden in the world. It is estimated that there are 845, 000 new TB cases each year, with a mortality rate of up to 98, 000 cases or the equivalent of 11 deaths/hour. In 2019 the number of TB cases in Indonesia was 543, 874, a decrease compared to all TB cases found in 2018, which amounted to 566, 623. The highest number of cases were reported from provinces with large populations, namely West Java (123, 021), East Java (65, 448), and Central Java (54, 640). Tuberculosis cases in these three provinces account for almost half of the total number of TB cases in Indonesia (45%) (Kemenkes, 2020). The transmission and development of TB disease are increasingly widespread because it is influenced by social factors such as poverty, urbanization, a less active lifestyle, use of tobacco and alcohol (Perpres, 2021).

To stop the global TB epidemic, WHO introduced The End TB Strategy, which has been effective since 2016. In connection with this strategy, targets have been set related to the SDGs (Sustainable Development Goals) for countries, namely reducing the number of TB deaths by 90% and the number of cases of New TB by 90% in 2035 (WHO, 2020). The Government of Indonesia is committed to quickly

achieving TB elimination by 2030. One of its strategies is to provide quality services in the management of TB provided by health care facilities in the region, namely the availability of policies, early detection, diagnosis, and management of TB that are comprehensive and integrated and the availability of diagnostic facilities that are sensitive and specific for TB disease that can be accessed by the whole community (Perpres, 2021).

In general, the examination of *Mycobacterium tuberculosis* uses the conventional method of direct sputum smear and culture. Conventional methods are less sensitive, so they can only detect half of active TB. Culture is the gold standard method, but it requires laboratory technician skills and takes much time to get results (Husna & Novi, 2020). The rapid molecular test is a molecular inspection method based on repeated amplification of the target DNA and then detected fluorometrically using a PCR instrument (Sudha, 2016). Considering the importance of high accuracy of diagnostic tools in Indonesia, the rapid molecular test (Xpert MTB/RIF) has been introduced in several regions (Kemenkes, 2017). The molecular rapid test tool allows doctors to diagnose TB in less than two hours with high accuracy, including TB that is already resistant to rifampicin (Litbangkes, 2018). Xpert MTB/RIF is a new laboratory test method, an automatic method, in the form of a cartridge or package with reagents for DNA amplification reactions to detect *Mycobacterium tuberculosis* bacteria and sensitivity to anti - TB drugs within 2 hours (Mertaniasih, 2020). Another molecular technique with an isolated isothermal polymerase chain reaction (iiPCR) method in the portable device POCKIT™ can be used as an alternative approach to Xpert MTB/RIF. iiPCR Pockit TB detection results are shown as positive and negative based on the fluorescence ratio after/before reaction with 85 minutes processing time (Chou et al., 2011).

Comparative tests between microscopic examination of TB and TCM Xpert MTB/RIF have been carried out frequently, while comparisons between Xpert MTB/RIF with the iiPCR Pockit TB method are still very rare, so it is considered necessary to conduct research to find out whether the iiPCR Pockit TB method is comparable to Xpert MTB/RIF in detecting *Mycobacterium tuberculosis*. The global challenge for equipment modernization can be in the form of transfer of examination technology and needs to be introduced as an alternative examination equipment in health care facilities, one of which is a hospital. Molecular diagnostic tools can help in accelerating TB detection. With early diagnosis, it is hoped that it can increase the scope of new case detection and follow - up and can support the National TB Control Target in accordance with the global elimination target, namely TB Elimination in 2035 and Indonesia free of TB in 2050.

Dr. Chasbullah Abdulmadjid Hospital is one of the government hospitals in Bekasi City which has integrated pulmonary services including inpatient, outpatient, poly DOTS and laboratories for TB sputum examination and TB molecular rapid test. In improving modern technology - based services, it must be supported by sophisticated and modern equipment, one of which is molecular tools. In the Laboratory of dr. Chasbullah Abdulmadjid Hospital provided the Xpert MTB/RIF and iiPCR Pockit TB machine, but a comparison of the results between the two had never been carried out. With the existence of this new tool, it is necessary to compare the results with existing equipment, hence this research is conducted.

2. Problem Definition

This study focuses on comparing the results of examination Xpert MTB/RIF dengan iiPCR Pockit TB in Tuberculosis suspects.

3. Methodology

The research isan analytical observational study with cross - sectional approach in which the variables was examined only once at a time to compare the results of the Xpert MTB/RIF and the iiPCR Pockit in TB suspects' patients. This research was conducted at the dr. Chasbullah Abdulmadjid Hospital in Bekasi City from January – June 2022. Ethically, this research has been deemed clear, obtaining approval from Ethic Committee of dr. Chasbullah Abdulmadjid Hospital No.015/KEPK/RSCAM/VI/2022.

3.1 Population and sample

The population in this study were all TB suspects patients. The sample in this study was sputum samples of TB suspects' patients in dr. Chasbullah Abdulmadjid Hospital from April to June 2022. A total of 47 sputum that met the inclusion criteria was consecutively sampled. It is calculated using a special sample size formula for conformity testing. The inclusion criteria used were the sputum of TB suspects' patients who have never received the treatment having good quality such as being purulent, greenish yellow in color, volume 2 - 4 ml, and slightly thick consistency. Meanwhile, sputum samples containing saliva and/or leftover food were excluded.

3.2Research procedure

The sputum samples were examined following the protocol of Xpert MTB/RIF (Cepheid, 2015) and iiPCR Pockit TB of Genereach Taiwan machine from the manufacturer.

3.3Data analysis

The collected data were statistically analyzed using cross - tabulations and the Kappa test. The degree of concordance between Xpert MTB/RIF and iiPCR Pockit TB was analyzed by Cohen's Kappa coefficient (K).

4. Results & Discussion

Comparison between results of XpertMTB/RIF and iiPCRPockitTB of the 47 samples at dr. Chasbullah Abdulmadjid Hospital in Bekasi City obtained results as following:

Table 4.1: Result of Xpert MTB/RIF and iiPCRPockitTB

Result	XpertMTB/RIF		iiPCRPockit	
	N	%	N	%
Positive	38	80.9%	37	78.7%
Negative	9	19.1%	10	21.3%
Total	**47**	**100.0%**	**47**	**100.0%**

Table4.1 shows results positive on the Xpert MTB/RIF as many as 38 (80.9%) whereas on iiPCRPockitTB37 (78.7%) positive results were obtained. Xpert MTB/RIF detects more than iiPCR PockitTB, in which related to the Pockit Central *Mycobacterium* detection limit of TB Premixes Reagents thatisaround10copy/reaction. This reagent can detect the TB complex, and does not detect other pathogens, such as *non - tuberculosis Mycobacteria* (NTM). According to research conducted by Rivani et al. (2019), the sensitivity and specificity of Xpert MTB/RIFis97% and 62% respectively.

Table 4.2: Statistical Analysis of Xpert MTB/RIF and iiPCR PockitTB Result

Results		XpertMTB/RIF		Total
		Positive	Negative	
iiPCRPockit TB	Positive	36	1	37
		76.6%	2.1%	78.7%
	Negative	2	8	10
		4.3%	17.0%	21.3%
	Total	38	9	47
		80.9%	19.1%	100.0%

Symmetricmeasures				
	value	*asymp. std. error*	*approx. Q*	*approx. sig.*
Measure of Kappa agreement	0.802	0.109	5, 512	0.000
Nofvalidcases	47			

Table 4.2 shows that there is agreement in the results between both instruments. Of the 47 samples analyzed, there were 44 (93.6%) samples that are considered consistent, comprising 36 (76.6%) samples with positive results and 8 (17.0%) samples with negative results. Consistency of results related to the similar principles of both instruments, that is detection of nucleic acids based on PCR. Nevertheless, there are3 (6.4%) samples showing different results.2 (4.3%) sample detected positive on XpertMTB/RIF and not detected on iiPCRPockitTB, while1 (2.1%) sample not detected by XpertMTB/ RIF but detected positive by iiPCRPockit TB. Different results are related with sample homogenization because more specimen formerly checked to be used for XpertMTB/RIF and the remaining specimens were used for iiPCR Pockit TB examination, in which sample stability after adding buffer is 4 hours. The results of the analytical study show that the Xpert MTB/RIF assay had analytic sensitivity for five copies of pure DNA genome, and131CFU/ml whereas detection limit of iiPCR PockitTBis10copy/reaction.

Agreement is the size of variable with two different testing to produce similar results (Flight et al., 2015). Measurement of consistency between the two methods, XpertMTB/RIF and iiPCRPockit TB, was carried out with agreement analytical statistic of KappaCohen (Zaki, 2012). Coefficient Kappa Cohen can be used to measure agreement of alternative method with the existing method (Anonymous, 2022). Statistics Kappa used to produce estimation reliability between two evaluators on results categorical or ordinal (Heidel, 2022). Strength agreement the will interpreted as bad (K≤0.20), enough (K0.21 - 0.40), moderate (K0.41 - 0.60), good (K0.61 - 0.8), and very good (K≥0.81) (McHughs, 2012).

Statistical analysis in table 4.2 shows the n value coefficient KappaCohenof0.802. This means that there is good agreement between XpertMTB/RIF and iiPCR Pockit TB in detecting *Mycobacterium tuberculosis*. Asymptotic value standardized error of 0.109 indicates a standardized error measurement in which the smaller value signifying the more reliable. The probability value obtained is0.000 (p - value ≤0.05), where concluded that there is significant difference in the results of *Mycobacterium tuberculosis* examination between XpertMTB/RIF and iiPCR PockitTB.

Some advantages of the iiPCR Pockit TB include minimal sample pretreatment, as well as the availability of lights UV built - in for decontamination. Besides, the reagents are lyophilized to facilitate delivery in room temperature and storage during testing (Chang et al., 2012; Tsai et al., 2012). Furthermore, iiPCR Pockit TB is easy to use infield with tool dimensions (31 x 26 x 15cm; 2, 1kg) convenient to move as well as results readily displayed on the instrument's monitor. This is different from the Xpert MTB/RIF, which requires a separate computer device to display the results. Detection time with the iiPCR Pockit TB is shorter (85 minutes) than the XpertMTB/RIF (1 hour 45 minutes).

There are limitations of the iiPCR Pockit TB, in which one of them is the qualitative system without quantitative module. This disables absolute quantification of the detected pathogen. However, the optical system is similar to the machine of real time PCR, meaning it has potential to be upgraded to a quantitative system in the near future. Neither does the testing distinguish between living and dead microorganisms. Nevertheless, as an alternative method to initial screening, the ii PCR Pockit TB is acceptable. To properly use the iiPCR Pockit TB as an initial screening, it is necessary to carry out further research related to discrepancy results between Xpert MTB/RIF and iiPCR Pockit TB as identified in this research. This is important because it is closely related to the diagnosis of TB which affects the accuracy of treatment. The aspects to be researched can range from the clinical aspects of the patients to the sample preparation.

5. Conclusion

The comparison between Xpert MTB/RIF and iiPCR Pockit TB in the examination of TB suspect patients reveals consistency in result but minor discrepancy is still noted.

6. Future Scope

To properly use the iiPCR Pockit TB as an alternative method for initial screening, it is necessary to carry out further research related to discrepancy results between Xpert MTB/RIF and iiPCR Pockit TB in which may relate with clinical aspects of the patients as well as the sample preparation.

References

[1] Anonim.2022. Uji Konsistensi Cohen's Kappa. Pelatihan Statistik Universitas Indonesia. https: //pelatihan - ui. com/uji - konsistensi - cohens - kappa
[2] Badan Penelitian Dan Pengembangan Kesehatan Kementerian KesehatanRI.2018. Laporan Penelitian Studi Evaluasi Deteksi Kasus TBC Dengan Tes CepatMolekuler (TCM) Di IndonesiaTahun 2018.
[3] *https: //e - riset. litbang. kemkes. go. id/download. php?file=1. %20Laporan - 2018 - Pusat%203 - Studi%20Evaluasi%20Detek. pdf*
[4] Cepheid®. Xpert® MTB/RIF package insert ref GXMTB/ RIF - US - 10. 2015; (Feb): 1 - 48. Availablefrom: URL *http: //www.cepheid. com/manageddownloads/xpert - mtb - rifenglish - package - insert - 301 - 1404 - rev - b - february2015. pdf.*
[5] Chang, H. G., Tsai, Y., Tsai, C., Lin, C., Lee, P., Teng, P., etal.2012. A Thermally BaffledDeviceForHighly Stabilized Convective PCR. Biotechnology journal, 7 (5) 662 - 666. Doi: 10.1002/biot.201100453.
[6] Chou WP, Chen PH, Miao M Jr, Kuo LS, Yeh SH, Chen PJ.2011. Rapid DNA amplification in a capillary tube by natural convection with a single is othermal heater. Biotechniques. p; 50: 52–7.
[7] Heidel.2022. Kappa. A very conservative measure of inter - raterreliability. *https: //www.scalestatistics. com/kappa. html*
[8] Flight, L., &Julious, S. A.2015. The disagreeable behavior of the kappastatistic. Pharmaceutical Statistics, 14 (1), 74–78. *https: //doi. org/10.1002/pst.1659*
[9] Kemenkes RI.2017. *Petunjuk Teknis Pemeriksaan Tuberkulosis MenggunakanAlatGeneXpert.* Direktorat Jenderal Bina Upaya Kesehatan. Jakarta.
[10] KemenkesRI.2020. Profil Kesehatan IndonesiaTahun2019. URL: *https: //pusdatin. kemkes. go. id*
[11] Kemenkes RI.2020. PedomanNasional Pelayanan Kedokteran. *Tatalaksana Tuberkulosis.* Direktorat Jenderal Pencegahan dan Pengendalian Penyakit. Jakarta.
[12] McHughs, M. L.2012. Interrater Reliablity: the KappaStatistic. Biochemia Medica, 22 (3), 276–282. Retrieved from. *https: //www.ncbi. nlm. nih. gov/pmc/articles/PMC3900052*
[13] Mertaniasih, NiMede.2020. TesCepatMolekulerXpertMTB/RIFuntukPenegakanDiagnosisTBC: Antara Canggihdan Kendala Pelaksanaan. UNAIR News. April 28, 2020. *https: //www.sciencedirect. com/science/article/pii/S2405579420300188*
[14] HusnaN., NoviU.2020. Perbandingan Hasil Pemeriksaan Mikroskopis Basil TahanAsamMetode DekontaminasiDengan Metode TesCepatMolekuler.
[15] Peraturan Presiden RI No.67 tahun 2021 Tentang Penanggulangan TuberkulosisPerhimpunan DokterParuIndonesia.2011. PedomanDiagnosis danPenatalaksanaandiIndonesia. Jakarta.
[16] Rivani E., Sabrina T., Patricia V.2019. Perbandingan Uji Diagnostic GenexpertMTB/RIF Untuk Mendeteksi Resistensi Rifampicin *Mycobacteriumtuberculosis*padaPasienTBParudiRSUP dr. Moh. HoesinPalembang. JKK, Volume6, No1, Januari2019: 23 - 2823p - ISSN2406 - 74 31; e - ISSN2614 - 0411.
[17] Sudha S.2016. Tuberculosis Diagnosis - An Overview To The Conventional Diagnostic Methodology and Need For Nano diagnosis. IntJMedEngInform.2016; 8 (1): 27 - 40. doi: 10.1504/IJMEI.2016.073664
[18] *URL: https: //doi. org/10.1371/journal. pone.0152694*
[19] TsaiYL, WangHT, Chang HF, etal.2012. Development Of Taqman Probe – Based Insulated IsothermalPCR (iiPCR) For Sensitive And Specific On – Site Pathogen Detection. PLoSOne.2012; 7 (9): e45278. doi: 10.1371/journal. pone.0045278
[20] WorldHealthOrganization.2020*GlobalTuberculosis Report*. Geneva.
[21] Zaki, R., Bulgiba, A., Ismail, R., & Ismail, N. A.2012. Statistical Methods Used to Test for Agreement of Medical Instruments Measuring Continuous Variables in Method Comparison Studies: A Systematic Review. PLoS ONE, 7 (5), e37908+. *https: //doi. org/10.1371/journal. pone.0037908*