INTERNASIONAL CONFERENCE ON
MULTIDISCIPLINARY APPROACHES IN HEALTH SCIENCE
VOLUME 1 TAHUN 2023, ISSN 3032-4408 (Online)
https://ejournal.poltekkes-denpasar.ac.id/index.php/icmahs

Identification Of Mycobacterium Tuberculosis With R-PCR (Real Time Polymerase Chain Reaction) Technique for Diagnosis Of Pulmonary TB Patients Balkesmas Semarang

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Article history
Posted : 2023-11-20
Reviewed : 2023-10-08
Received : 2023-09-26

ABSTRACT

Background: Tuberculosis is one of the causes of death due to infection and is transmitted globally by more than 10 million people. Pulmonary tuberculosis (TB) is an infectious disease which is a priority health problem in developing countries, including Indonesia. 16 countries accounted for 93% of tuberculosis cases with 3 countries contributing the most tuberculosis cases namely India, Indonesia and the Philippines. It is very important to develop methods to increase the sensitivity and specificity of TB examination results using the r-PCR (real time polymerase chain reaction) technique. **Methods**: This process requires a double-stranded DNA template containing target DNA, DNA polymerase enzyme, triphosphate nucleotides, and a pair primer.which has better accuracy and precision by using IS6 and IS7 primer. To determine the sensitivity and specificity of the R-PCR (REAL TIME POLYMERASE CHAIN REACTION) TECHNIQUE method. This research is non-experimental analytic in nature. The sample used in this study was a pure culture of Mycobacterium tuberculosis growth in the National Health Service Office of Central Java Province with microscopic testing AFB staining, R-PCR (REAL TIME POLYMERASE CHAIN REACTION). **Results**: The results showed that there was no difference between the conventional culture identification method as the standard for identification of Mycobacterium tuberculosis and the results of RT PCR examination with IS6110 primer having 100% sensitivity and specificity.Our conclusion is that the is6100 primer can be used as a primer to detect Mycobacterium tuberculosis

Keywords: Mycobacterium tuberculosis, Kultur , R–PCR

INTRODUCTION

Pulmonary tuberculosis (TB) is an infectious disease which is a priority health problem in developing countries including Indonesia, TB (Tuberculosis) is an infectious disease usually caused by bacteria (Mycobacterium tuberculosis), which mostly attacks the human lungs (Wedari et al., 2021, Nurjana et al., 2020,Ullah et al., 2021). Tuberculosis is one of the causes of death due to infection and transmission globally, more than 10 million people are infected with 1.4 million deaths from tuberculosis and 465,000 cases of drug resistance, 16 countries account for 93% of tuberculosis cases with 3 countries contributing the most tuberculosis cases, namely India. , Indonesia, Philippines In the last ten years, tuberculosis cases have decreased by almost a third. Mycobacterium tuberculosis infection is resistant to the Anti-Tuberculosis Drugs (OAT) isoniazid and rifampicin and to other OATs known as multidrug-resistant TB (MDR TB). (World Health Organization , 2021., Parums, 2021.,Dheda et al., 2022.,Hatami et al., 2022).

Cases in Indonesia are estimated to be 824,000 confirmed Tuberculosis with 393,323 cases of RR TB and 7,921 MDR TB (Indonesian TB, 2022). Tuberculosis cases in Central Java were 114.60 per 100,000 population. The city with the highest contribution to Tuberculosis findings was Tegal with 762.10 per 100,000, Magelang City with 507.30 per 100,000 and Banyumas 205.90 per 100,000. (BPS Central Java, 2022) The State of Indonesia implemented the *Mycobacterium tuberculosis* identification procedure using the Rapid Molecular Test method to detect mutations in the rpo B gene, mutations in the rifampicin drug target gene, which must be confirmed with a drug susceptibility test (DST) which requires examination time for the Mycobacteria Growth Indicator Tube (MIGT) method, between 7 days. up to 21 days and the conventional Lowenstein Jensen method takes 6 weeks to 8 weeks.

So far, in the diagnosis of TB disease, three different methods have been used to determine the presence of *M.tuberculosis* infection, although each has advantages and limitations. This method of direct identification of acid-fast bacteria with a microscope is very fast, but less sensitive because the sample must contain a minimum of 104 cells/per ml. The culture method can produce 100% growth, if the sample is positive, Mycobacterium on the culture medium is very slow, which is between 4-6 weeks. The way the Rapit Test Ag MTb detects is very practical for use in the clinic, but the drawback is that it is less sensitive and less specific. It is necessary to develop a method for determining Mycobateria in clinical samples that are sensitive and fast. PCR is a way of amplifying DNA, in this case *Mycobacterium tuberculosis* DNA, in vitro. This process requires a double-stranded DNA template containing target DNA, DNA polymerase enzymes, nucleotide triphosphate, and a pair of primers that have better accuracy and precision. This study aims to analyze the results of specific PCR primers for the identification of tuberculosis

METHOD

This research was conducted at the Central Java Provincial Health Laboratory and the Biology Campus, Health Analyst Department, Semarang Poltekkes. The population in this study were patients who were diagnosed with TB at Balkesmas Semarang on a specified date for 1 month. This research is a diagnostic test

designed in cross sectional way. The study was conducted on pulmonary TB patients at Balkesmas Semarang. With the criteria of initial diagnosis before treatment with criteria of age 15-50 years whose sputum samples have been tested and the results of the diagnosis are known to represent predetermined research data, the Ziehl Neelsen Staining Method. Then it was observed using a microscope and the results were determined according to the standards of the International Union Against To Lung Disease. in our research process we have fulfilled the ETHICS DESCRIPTION, this research does not interfere with patients, this research only takes some sputum samples from the BALKESMAS laboratory diagnostic process. This research complies with laboratory operational standards and safe procedures in the research process.(IUATLD, 2000).(Widodo & Pramono 2017.,Widodo & Priyatno 2020)

Mycobacterium tuberculosis culture with Lowelstein Jensen media, samples were decontaminated with 10% NaOH, washed, supernatant was removed, 100 µl of sediment was inoculated on LJ media. Incubate again at 37^0C in an upright position with the lid tightly closed, for 4-8 weeks. Observations were made every week, biochemical tests identified Mycobacterium tuberculosis. RT PCR examination. Tuberculosis-specific primers targeting the IS6110 gene with primers IS6: GGC TGT GGG TAG CAG ACC and IS7: CGG GTC CAG ATG GCT TGC. Denatured PCR cycle at 95 0C for 5 minutes, cycle 40 times at 95 0C and 60 0C for 1 minute. IS6 and IS7 primers are specific primers for the *Mycobacterium tuberculosis* complex group including *Mycobacterium africanum, Mycobacterium canettii, Mycobacterium bovis, Mycobacterium tuberculosis. For the Non-tuberculous mycobacteria (NTM) group including Mycobacterium abscessus, Mycobacterium africanum, Mycobacterium avium, Mycobacterium kansasii, Mycobacterium leprae, Mycobacterium marinum*(Desjardin et al., 1998).

RESULTS

A. In this study, 20 sputum samples were used which had been microscopically examined for bacteria with Ziehl Neelsen staining with positive results, then cultured with Lowenstein Jensen's media to obtain results according to the table below:

Table: 1 Table of Microscopic Test and Culture

Microscopic test of sputum samples	Culture Test Culture		Number of samples
	Positive	Negative	
Positive	20	0	20
Negative	0	0	0
Number of samples	20	0	0

Table : 2 Conventional identification compared to PCR identification with IS6 and IS7 primers

BIOCHEMICAL IDENTIFICATION TEST	PCR Primer (IS1)		Number of samples
	Positive	Negative	
Positive	18	0	18
Negative	0	2	2
Number of samples	18	2	20

Test results of Mycobacterium tuberculosis isolate culture from the Health Laboratory and Medical Devices Testing Center. 20 test samples obtained positive results, 18 samples were identified as Mycobacterium tuberculosis and 2 samples were identified as Mycobacterium other than tuberculosis (MOTT)

DISCUSSION

The results of laboratory examination on the test samples obtained positive results, 20 positive smear microscopic samples and culture was carried out with the growth results. A direct test was carried out by making preparations, then ZN painting was carried out, the culture process was carried out with NaOH decontamination, then planted in LJ media for 5 week, observed every week, after growing then tested for P nitrobenzoic acid (PNB), niacin and hot catalase to determine the bacterial subtype. M. tuberculosis was incubated at 37^0C for 5 weeks, if there was no growth, continued incubation for up to 8 weeks, if there was no growth, it was reported as negative, if there was a change in color from light blue to dark green with non-acidic bacteria, with fast growth it was said to be contamination(Kassaza et al. 2014.,Holani et al. 2014) In this study, 20 culture samples were subjected to Niacin and nitrate biochemical tests. To identify these samples, 18 samples included Mycobacterium tuberculosis and 2 samples included the Non-tuberculous mycobacteria (NTM) group. Based on these results, a follow-up examination was carried out using the PCR technique. Mycobacterium tuberculosis as a member of the genus Mycobacterium has non-motile growth characteristics, does not form spores, straight rod-shaped cells with a length of 2-4 µm and a width of 0.2-0.5 µm, has a cell wall shape similar to Gram positive but has a thick lipid layer, in liquid media its growth is pleomorphic morphologically can be in the form of single rod cells or as multicellular and branching filaments with a generation time of > 24 hours, while avirulent species such as *Mycobacterium smegmatis* grow rapidly with a generation time of > 3-4 hours. Mycobacteria which are aerobic and pathogenic in mammals consist of 7 different species namely M. tuberculosis, M. bovis (ssp.bovis and caproe), M. aficanum, M. microti, M. conetti, M. pinpipedi .(SHARMA ,2020.,Gharbi et al. 2019)

Test results of *Mycobacterium tuberculosis* isolate culture from the Health Laboratory and Medical Devices Testing Center.

20 test samples obtained positive results, 18 samples were identified as Mycobacterium tuberculosis and 2 samples were identified as Mycobacterium other than tuberculosis (MOTT). IS7 obtained CT results between 8.78 to 19.62 indicating that 18 samples were positive for Mycobacterium tuberculosis while 2 samples with codes 1203 and 1265 obtained primary unreadable Ct results in this test with IS6: GGC TGT GGG TAG CAG ACC and IS7: CGG GTC CAG ATG GCT TGC. DNA sequencing of Mycobacterium tuberculosis with Priem 16S rRNA with yields of 99.9% at the MTBC nucleotide level included M. tuberculosis, M. africanum, M. canneti, M. microti, and M. bovis. Members of the complex are also known to grow slowly with a doubling time ranging from 12 to 24 hours which is of course influenced by the nature of the pathogen and environmental factors. Several factors influencing genetic variation are caused by differences in location and number of copies of the insertion sequence specific forms of M. bovis and M. tuberculosis (IS 6110). Furthermore, slight genetic change might be caused by differences in polymorphic GC-rich sequences (PGRS).(Coscolla & Gagneux 2014.,Embden et al. 1993)

Mycobacterium tuberculosis K from the Beijing family has a gene sequence length of 4,385,518 bp complete genome (one chromosome) with a G + C composition of 65.59% consisting of 4194 genes (3447 function genes, 48 RNA genes (3rRNA and 45tRNA) and 216 genes peptide [40] This study attempted to compare the results of the examination between the identification of Mycobacterium tuberculosis culture and the results of the PCR examination of 20 samples of the 100% sensitivity test and the 100% specificity test showing that the results of the examination between the Conventional and the PCR test showed no difference indicated by the statistical test with the value sig > 0.05, i.e. 1.0 so that HO is accepted there is no difference between the results of conventional tests and qRTPCR.The use of molecular methods can be used as an alternative to detect *Mycobacterium tuberculosis* quickly and precisely.

CONCLUSION(S)

The results showed that there was no difference between the conventional culture identification method as the standard for identification of *Mycobacterium tuberculosis* and the results of RT PCR examination with IS6110 primer, which had a sensitivity and specificity of 100%.

Conflict of Interest

I have no conflict of interest in this research, we are focused on understanding the use of PCR technology for education

Acknowledgment

Thanks to the Director of the Health Ministry Polytechnic of Semarang, the Semarang Ministry of Health Polytechnic DIPA which has funded the research, Elly Karina, Nur Jani, Tri Sugiatmini who has assisted the research process.

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