ABSTRACT

Diagnostic of Mycobacterium tuberculosis with Technique of Zhiel Neelsen & PCR (Polymerase Chain Reaction) and Resistance by MTB / RIF Genexpert Technique, 2017.

Tuberculosis (TB) is the most common cause of death in developing countries of which 75% of patients are in the productive age of 20-49 years, because in developing countries have dense population and high prevalence, so more than 65% cases of tuberculosis occur in Asia. Terjangsun increase in this case is caused by the immune system, nutritional status and personal hygiene of individuals and density of residential neighborhood. WHO recommends treatment for Pulmonary TB patients with DOTS strategy (Directly Observed Treatment Shortcourse Chemotherapy) or direct supervision of short-term and daily medication only 36% with an 87% cure rate. Due to the irregular treatment and inadequate combination of drugs in the past there may be immunity of TB germs against widespread or multi drug drug resistance (MDR). This study aims to determine the resistance and sensitivity of Mycobacterium tuberculosis to Anti Tuberculosis drug in tuberculosis patients with PCR and Genexpert technique. The research used laboratory experimental method which was conducted in laboratory of Microbiology Faculty of Medicine and Health Sciences of Syarif Hidayatullah State Islamic University Jakarta in May 2017. The method used was identification of bacteria with Zhiel Neelsen staining, Homogenase and Decontaminase, Biochemical Test of Mycobacterium tuberculosis, PCR, and GenexpertMTB / RIF. Sputum sampling is done in the morning - while (SPS). The result of BTA staining from Bojonggede Puskesmas was 84 samples, BTA positive was 35 samples and the percentage of BTA positive was 42%, while BTA negative was 49 and the percentage was 58%. PCR test results from 20 samples was 14 positive samples that mean people with tuberculosis infection 70%, while the negative is 6 samples means people who do not suffer from tuberculosis by 30%. The conclusion of this research is PCR test result compared with result of BTA test with Zhiel Neelsen, that is the result of positive test of the percentage is bigger than smear staining test result.

Keywords: Mycobacterium tuberculosis, mutation, multi drug resistance (MDR), decontamination.
INTRODUCTION

A. Background

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis and was discovered over 100 years ago. Until now tuberculosis is still a serious problem worldwide, as it is the leading cause of death. Every year an estimated 8 million new infections occur and 2.5 to 3 million cause death (Zhang et al., 2005). Of all cases of tuberculosis in the world, most occur in developing countries, including Indonesia is ranked third after the state of India and China, with the number of new cases about 539,000 cases and the number of deaths about 101,000 people per year. According to the Ministry of Health (2008), the disease is most prevalent in developing countries of about 75% of patients are at productive age of 20-49 years, this is because in developing countries have a dense population and a high prevalence, resulting in more than 65% of cases Tuberculosis occurs in Asia. Based on Household Health Survey (SKRT) in 1992, pulmonary tuberculosis in Indonesia was ranked second after cardiovascular disease.

According to WHO (2010) states that Indonesia every year there are 583 new cases with the death of 130 patients with positive tuberculosis on dahaknya. Hasil research Kusnindar (1990) the number of deaths caused by tuberculosis estimated 105,952 people per year. The incidence of high pulmonary tuberculosis cases is most
prevalent in socioeconomic groups. The increase in cases is influenced by immune
system, nutritional status and personal hygiene of individuals and density of residential
neighborhood.

In 2011 WHO recommends treatment for Pulmonary TB patients with DOTS
strategy (Directly Observed Treatment Shortcourse Chemotherapy) or direct daily
swallowing of short-term medication only reaches 36% with an 87% cure rate. Prior to
the DOTS strategy (1969-1994) the coverage was 56% with a cure rate of only 40-60%.
Because irregular treatment and inadequate drug combinations in the past may have
arisen TB germs against OAT (anti-tuberculosis drugs) widespread or multi drug
resistance (MDR).

Conventional laboratory tests for TB diagnosis and drug sensitivity testing to
detect first-line and second-line drug resistance take a long time to know the results and
with more complicated techniques (Boehme, 2009; WHO, 2013). While the patient is
waiting for the diagnosis, the patient's illness worsens and the patient can move the
OAT resistant tuberculosis disease to another, especially to the family member. Early
case detection of MDR TB is important to inhibit transmission and to prevent further
spread of MDR TB so that new diagnostic tests are needed (Boehme, 2009).

Conventional tests include the method of seed culture culture, aimed to
multiply the bacteria Mycobacterium tuberculosis in sputum specimens, so as to
improve the detection of sensitivity. Currently there are several media that can be used
as cultures from Mycobacterium tuberculosis, such as solid and liquid media, such as
Lowenstein-Jensen, Mycobacteria Growth Indicator Tube (MGIT) and microcolonial
culture. But all the above checks cost not cheap. Thus, other techniques in kultr research
are still developing to detect Mycobacterium tuberculosis in order to obtain cheap
culture method and high sensitivity and specificity. A diagnostic test of the culture of the medium taken to identify the Mycobacterium tuberculosis bacteria has some disadvantages including it takes longer to get the results, although the cost can be cheaper (Indah W, 2010).

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According to WHO (2011 & 2010) states that only a small portion of the 440,000 MDR-TB sufferers have adequately tested drug sensitivity. The most important problem is that conventional diagnostic methods are currently slow and complicated (O.Grady et al., 2011; WHO, 2008). First required isolation of Mycobacterium tuberculosis (M. tuberculosis) on the specimen, then identified, and continued with the OAT sensitivity test. The test of this recommended World Health Organization (WHO) is a method of proportion using media such as Lowenstein Jensen, which takes 8- 12 weeks. During that time the patient received an inappropriate drug and an increase in the number of M. mutant tuberculosis (WHO, 2008 & Chiang CY, 2010).
According to Diana et al (2010) has conducted research with a diagnostic test designed by cross sectional. Sputum examination of patients is done by three examination techniques, namely by PCR techniques, examination of BTA microscopically, and bacterial culture. The results of the study by comparing the method of examination of Acid Resistant Bacteria microskospik with PCR technique to detect Mycobacterium tuberculosis has a sensitivity of 30%, specificity 80%, and accuracy of 47%. The significance test with Mc Nemar yielded significant differences. (p <0.01). While the comparison of TB bacterial culture method with PCR technique to detect Mycobacterium tuberculosis has 65% sensitivity, 40% specificity, and 57% accuracy. The significance test with Mc Nemar gave no significant difference. (p = 1.0). And the comparison of microscopic acid bactericidal examination with bacterial culture method of tuberculosis to detect Mycobacterium tuberculosis have sensitivity 31.6%, specificity 81.8%, and accuracy 50%. The significance test with Mc Nemar yielded significant differences. (p <0.01). Thus, it can be concluded that detection of Mycobacterium tuberculosis by PCR technique is as good as bacterial culture of tuberculosis, but the time of examination with PCR technique is shorter than culture culture of Mycobacterium tuberculosis much undetectable by microscopic examination (BTA).

According to Maria Lina et al (2002), states that have conducted research on M. clinical isolate bacteria M. tuberculosis using Pt8 & Pt9 primer oligonucleotide by PCR method, which has ability to detect amount of DNA equal to 100 fg equal to 20 bacterial cell.

According to Maria Lina (2006) reported that the Nested PCR method can detect M. tuberculosis in 15 isolates among 20 clinical isolates while the other 5 isolates negative with nested-PCR turned out to be 4 isolates classified as MOTT and 1 non-
mycobacteria isolate. The M. tuberculosis germ on 21 sputum samples from 30 samples consisting of 25 samples of AFB + and 5 smear samples -, can be detected with nested PCR. Nested PCR using primers designed from the rpo ~ M. tuberculosis gene section is a fast, sensitive and specific method for detect both M. tuberculosis clinical isolates and direct clinical specimens such as sputum.

According to Maria Lina (2007) reported also that the Nested PCR method is more specific than culture in detecting M. tuberculosis. Nested PCR is more sensitive and specific than microscopic examination to detect M. tuberculosis. Nested PCR by using a primer designed from the rpo ~ M. tuberculosis gene section is a fast, sensitive and specific method for detecting M. tuberculosis and its resistance to the clinical isolates direct clinical specimens such as sputum.

Based on the description above, the research is done in an effort to find a fast, precise, specific and sensitive diagnostic technique to detect Mycobacterium tuberculosis bacteria with BTA stain with Zhiel Neelsen method and PCR technique which is one of the alternative diagnostics.

LITERATURE REVIEW

A. Morphology of Mycobacterium tuberculosis

Bacteria Mycobacterium tuberculosis has a size of 0.5 - 4 microns x 0.3 to 0.6 microns with a thin stem, straight or somewhat crooked, granular or has no sheath, but has a thick outer layer consisting of a lipoid called micolat acid (Widoyono, 2002 and Nofriyanda, 2010).
These bacteria include orders Actinomycetales, familia Mycobacteriaceae and genus Mycobacterium. Genus Mycobacterium has several species including Mycobacterium tuberculosis that cause infection in humans. Tubular bacillus is a straight slim, but sometimes slightly curved, shape with a length of 2μm-4μm and a width of 0.2μm-0.5μm. The organism is immobile, does not form spores, and is not encapsulated, when stained will look like a bead or granular. This bacterium is aerobic obligate and its growth is slow. It takes 18 hours to multiply and growth in ordinary culture media can be seen within 6-8 weeks (Jawetz et.al, 2005 and http://repository.usu.ac.id/bitstream/123456789/19500/4/Chapter%20II.pdf)

The growth of this bacteria grows optimally at 37°C and pH 6.4 - 7.0. If heated at 60 ° C the bacteria will die for 15-20 minutes, the bacteria are very sensitive to sunlight and ultraviolet radiation. Besides this organism is somewhat resistant to chemicals and resistant to drying, making it possible to stay alive for long periods in the spaces, blankets and cloths in the bedroom, sputum. This bacterium has a cell wall that consists of 60% of fat complexes such as mycolic acid that causes germs are acid resistant, cord factor is
mycosides associated with virulence. Bacteria that are virulent have a distinctive form called serpentine cord, Wax D which plays a role in immunogenity and phosphatides that play a role in the process of necrosis kaseosa. The tuberculosis bacillus is difficult to stain but once colored it will bind the strongly irreversible dye with an alcoholic acid solution such as Ziehl Nielsen stain. Such organisms are called acid resistant. Tuberculous bacilli can also be stained with fluorescence dyeing such as auramin rhodamin staining (Jawetz et al., 2005, Widoyono, 2002, and http://repository.usu.ac.id/bitstream/123456789/19500/4/Chapter%20II.pdf)

B. Tuberculosis epidemiology

New tuberculosis patients infectious lung in the world each year occurs about 4 million, coupled with patients who are not contagious. This means that every year in this world there will be about 8 million people with pulmonary tuberculosis, and there
will be about 3 million people died because of this disease. In 1990 there were more than 45 million deaths worldwide due to various causes, of which 3 million were (7%) due to tuberculosis. In addition, 25% of all actual deaths can be prevented due to tuberculosis. In 1990, Southeast Asia has seen 3.1 million new tuberculosis patients and more than one million deaths from this disease. In 2005 in Southeast Asia there were more than 8.8 million new tuberculosis patients and more than 1.6 million deaths (Jawetz et al, 2005, Tortora et al, 2010; http://repository.usu.ac.id/bitstream/123456789/19500/4/Chapter%20II.pdf).

C. Transmission and Spreading Tuberculosis

Transmission of tuberculosis by air by small particles containing a tuberculosis germ called "nuclear droplet". A 1-5 μm nuclear droplet can reach the alveoli. Droplets of small nuclei containing single bacilli are more dangerous than large amounts of bacilli in large particles, since large particles tend to accumulate the airway rather than to the alveoli so that they are excreted from the lungs by the mucosaliser system. Cough is the most effective mechanism for generating nuclear droplets. A quick, strong cough will produce as many infectious particles as five minutes of loud speaking. Airborne spread can also be caused by strong expiratory maneuvers such as sneezing, screaming, singing. One sneeze can produce 20,000 - 40,000 droplets, but most are large particles so they are not infectious. Patients who cough more than 48 times / night will infect 48% of the person in contact with the patient. While patients who cough less than 12 times / night infect 28% of their contacts (http://repository.usu.ac.id/bitstream/123456789/19500/4/Chapter%20II.pdf)

Bacterial tuberculosis bacilli enter the body through the gastrointestinal tract when drinking milk containing Mikobakterium tuberculosis. Other entrances into the human body are through injury to the skin or mucous membranes, but spreading in this way is very rare. If the focus of tuberculosis has been formed on one part of the body then the disease can spread to other parts of the body through blood vessels, lymphatic channels, direct contact, gastrointestinal tract (often from the intestinum back into the blood through the duct of the torasikus) and lastly the most frequently by the airway. (Jawetz et al, 2005; Tortora et.al, 2010; http://repository.usu.ac.id/bitstream/123456789/19500/4/Chapter%20II.pdf))
The risk of a person getting infected with this disease depends on the rate of exposure to sputum splash. Patients with pulmonary TB with positive smear give the possibility of greater transmission risk of lung tuberculosis patients with smear negative. The risk of transmission each year is demonstrated by Annual Risk of Tuberculosis Infection (ARTI), which is the proportion of people at risk of being infected with TB for one year.4.8 ARTI of 1%, meaning 10 (ten) people among 1000 people are infected each year. ARTI in Indonesia varies between 1-3%. (Jawetz et al; Tortora et al, 2010; http://repository.usu.ac.id/bitstream/123456789/19500/4/Chapter%20II.pdf)

D. Patogenesis: Mycobacterium tuberculosis

Tuberculosis is a disease controlled by cell mediated immune response. Effector cells are macrophages, while lymphocytes (usually T cells) are immunorespon se cells. Inhalation of large particles containing more than three tuberculosis bacilli will not reach the alveoli, the particles will be attached to the bronchial wall and will be excreted by the mucociliary system, but the inhalation of small particles containing 1-3 bacilli may reach the alveoli (Tortora et.al, 2010, http://repository.usu.ac.id/bitstream/123456789/19500/4/Chapter%20II.pdf and Jawetz et al, 2005).

Bacterial tuberculosis: Bacteria that infect the lung for 6 - 8 weeks will cause symptoms because it has activated T helper lymphocytes CD4 (cluster differentiated) to produce interferon gamma for macrophage activation so as to increase the ability of phagocytosis. In addition, TNF (tumor necrotizing factor) is produced by T lymphocytes and macrophages where TNF plays a role in macrophage and local inflammatory activation (Jawetz et.al, 2005; http://repository.usu.ac.id/bitstream/123456789/19500/4/Chapter%20II.pdf; Tortora et al, 2010).

Bacteria in the form of tubular bacilli that enter the alveoli will be followed by vasodilation and entry of polymorphonuclear leukocytes and macrophages that serve to eat and kill the bacillus. After a few days the leukocyte is reduced and the macrophages become dominant. The affected alveoli will be consolidated and symptoms of acute pneumonia, called primary focus or gonosis focus, are the primary infection. These primary infections can heal with or without scars or may continue and bacteria continue in phagocytes or multiply in cells. Basil can spread through the lymph
nodes to the regional lymph nodes. The combined aggravation of the lymph nodes with a primary focus is called the ghon complex. Primary infection occasionally persists and its pathological changes coexist as post primary primer TB. Primary post is commonly seen in the upper lung especially in the upper posterior segment of the upper lobe or in the lower apex part of the lobe. The occurrence of primary post TB can occur through any of these 3 mechanisms:

1. Direct development of primary TB
2. Reactivation of primary TB (endogenous)
3. Exogenous reinfection.

Successful bacteria enter the body, then there is the proliferation of tuberculosis bacilli in the central necrosis followed by the softening and liquefaction of the caseosa substances that can break into the bronchi and form the cavity. Bleeding may occur if the cationous process continues into the blood vessels of the cavity wall. Spreading of caseos and liquids into the branching of the bronchus will spread the infection to other lung areas. The rupture of the caseosa focus into the blood vessels will result in the occurrence of miliary TB (Tortora et al, 2010; Jawetz et al, 2005; http://repository.usu.ac.id/bitstream/123456789/19500/4/Chapter%20II.pdf).

Prevention of disease can be given by BCG vaccination which is active immunization where the vaccine used is the weakened bacteria that can not cause disease, but still can lead to immunity. Individuals who have been given a complete BCG vaccine then inside the body has formed an immune that can fight infection tuberculosis so although (Tortora et al, 2010 and http://repository.usu.ac.id/bitstream/123456789/19500/4/Chapter%20II.pdf).

E. Method / Technique of PCR (Polymerase Chain Reaction)

PCR is an in vitro method for enzymatically specific enzyme target DNA sequences by using a specific pair of oligonucleotide primers found in two known sequences. PCR was first discovered by Kary Mullis in 1983 and has been widely used in the molecular biology of molecular genetics, medical diagnosis, and forensic medicine (Kendrew, 1994). Basically, the PCR reaction takes the principle of DNA
replication, i.e., double strand opening, primary attachment, and extension of new DNA chains by DNA polymerase from 5’ to 3’ direction. (1997).

Components required in PCR reactions are thermostable enzymes Tag DNA polymerase, buffer buffer buffer solution, dNTP, template DNA, primary oligonucleotide, and cofactors such as magnesium (Mg2+). This mixture works repeatedly to multiply the target DNA chain, with the speed and specificity of the reaction, which is strongly determined by temperature. The success of this PCR reaction depends on efficient interaction with all of the above components (Kolmodin & Williams, 1989).

PCR process is a series of temperature cycles that occur repeatedly. One PCR cycle consists of 3 stages, namely: 1) denaturation, 2) annealing, and 3) extension. Denaturation is the process of separating a single strand of DNA into two single strands of DNA. This single-stranded DNA acts as a template (template), the primary attachment site, and the workplace of DNA polymerase. The separation of double strands of DNA can occur through a heating process that is generally carried out at 90-95°C for 30 seconds. Furthermore, at the annealing stage the reaction temperature is lowered to ~50°C for 30-60 seconds, for attaching primer oligonucleotides to a complementary sequence to a molded DNA molecule. The third stage in the PCR cycle is the extension performed at 72°C, which is the optimum temperature for the DNA tag enzyme polymerase work. This process lasts for approximately 1.5 minutes. Extension is a primary elongation process forming a complementary DNA sequence with a molded DNA. These three stages last for several times until the desired level of amplification. Generally the amplification lasts 25-40 cycles, depending on the amount of DNA desired. The steps of the PCR process are shown in Figure 3 (Snustad et al., 1997; Kendrow, 1994; Kolmodin & Williams, 1989).
Figure 3. The amplification process using PCR method. One cycle consists of denaturation, annealing, and extension. One PCR cycle will double the number of target DNA molecules. Each new chain that has been synthesized, will act as a mold for the amplification process. Furthermore each PCR cycle will multiply the DNA of the
template twice, so that the amount of DNA produced at the end of the PCR process is 
2^n (n = number of cycles) (Snustad et al, 1997).

RESEARCH METHODS

A. PLACE & TIME OF RESEARCH

This research will be conducted in the laboratory conducted in Microbiology 
Laboratory FKIK UIN Syarif Hidayatullah Jakarta, held in May to October 2016.

B. Research Methods

This research is a cross-sectional study with laboratory experimental method with Zhiel 
Neelsen and PCR staining method from sputum sample of tuberculosis patient for 
resistance test of Mycobacterium tuberculosis bacteria .

C. Population and Sample Research

Sputum samples from positive tuberculosis patients

Number of sputum samples that must be met in this study:

\[ n = Z^2 p q \]

\[ d^2 \]

\[ p = \text{proportion of bacteria Mycobacterium tuberculosis in population} = 9.5\% = 0.095 \]

\[ q = 1 - 0.095 = 0.905 \]

\[ Z = 1.96 \text{ (significance level) and} \ = 0.005 \]
d = 10% = 0.1

Then n = 33,028 33 sputum samples

D. Research Tools and Materials

1 Research Tools:

- Rotary evaporator
- Tube reaction
- Diluent thinner
- Vortex
- Objecck glass
- Water water
- Driver dish
- The timers

E. Materials Research

- Stable seades
- Fukhsin Carbol Color Zoom
- The methylene blue substance

Laboratory examination for Mycobacterium tuberculosis identification can be done in two ways: microscopic and PCR.

F. How it Works:

1. Microscopic examination

- The inspection material is made on a new and clean glass object. The dried preparation is fixed and stained by Ziehl Neelsen smear. After washing and dry it is checked under a 1000 X microscope with the help of immersion oil. Acidic Basil (BTA)
will appear rod, straight or crooked, individually or clustered, red on a blue base, then read according to IUAT (International Unit Against Tuberculosis) scale.

2. The Diagnostic Test of sputum samples by PCR method

A. Homogenization and Sputum DNA Extraction

Clinical isolates grown in Lowenstein Jensen media extracted their DNA by harvesting the bacterial isolates by adding a 0.9% NaCl solution. The extraction of DNA was done in the previous way (171) ie by melisis bacterial cells that have been harvested with 1 x TE (Tris-EDTA) solution, SDS (Sodium Dodecyl Sulfate) and K-proteinase. Then added phenol-chloroformisoamnic alcohol (24: 1) to extract DNA DNA precipitation is accomplished by adding ethanol and centrifugation at high speed. For PCR process, the obtained DNA pellet is then dissolved with a 1x TE buffer solution.

The process of homogenization and decontamination is performed for clinical specimens (sputum) in order to concentrate the sample so as to increase the number of bacteria especially M. tuberculosis contained in sputum samples and to eliminate microbes other than mycobacteria. Sputum is homogenized and decontaminated with acetyl-L-cysteine, NaOH and Naphthalis solution, then disenrifugated. The method for sputum DNA extraction is the Boom method (18). Cells dilute with Tris-HCl solution, guanidine thiocyanate as chaotropic agent, EDTA, and triton X-IOO. Diatom solution (DNA binder), acetone, ethanol 70% and high speed centrifugation are used for DNA extraction and precipitation. For PCR process, the extracted DNA is eluted with 1 x TE buffer.

B. Sample Preparation & DNA Extraction

A 1000-ul sputum sample was transferred to a 1.5 ml centrifuge tube, centrifuged at 12,000 rpm for 5 minutes, then taken as a template for DNA supernatant. Extracted DNA with QIAamp DNA (DNA code QQBB).

C. PCR Mix Preparation

Amplification of PCR enzymes HotStar DNA Polymerase. The reagent concentration for PCR reaction of 20 ul (each tube) consisted of 10 x PCR Buffer HotStar of 2 ul, 25mM MgCl2 of 0.8 ul, 10 mM dNTP Mix of 0.40 ul, 5xQ solution of 4 ul, 10 uM Primer PCRTB, HoStar DNA Polymerase 0.12 ul, Dnae-free water 8.10 ul. Then let
stand a few minutes to several hours. Then add PCR mix to 0.2 mL, then add DNA samples and vortex for 15 seconds.

- The DNA samples are fed into the PCR machine, with PCR reaction conditions being the initial denaturation at 95 °C for 15 minutes; denaturation 94°C for 30 seconds ;; 40 cycles, annealing at 60 °C for 30 seconds; elongation at 72 °C for 30 seconds; 40 cycles, and final elongation at 72°C for 5 min. The samples obtained were then electrophoresed and stored at -15°C to -20°C.

E. Electrophoresis and Documentation Process

- agarose gel electrophoresis techniques were used to analyze amplified DNA with a 1.5% (b / v) agarose concentration. Previously Added 3 ul 6x loading buffer (LB) into PCR mix tube.

- Then in the vortex for 15 seconds, centrifuged for 15 seconds. This process is carried out in a buffer buffer buffer buffer for 5 minutes.

- Coloring of electrophoretic DNA results was done by using a solution of ethidium bromide with concentration
RESULTS AND DISCUSSION

A. BTA Staining Result with Zhiel Neelsen Technique

Based on the result of BTA dyeing, samples from Bojonggede Puskesmas counted 25 times with 68 samples, the result showed 14 positive people and 11 negative people BTA, consist of 8 women and 6 men. This means more women suffering from tuberculosis than men. Distribution of cases by sex in patients with the highest lung TB positive results in this study was women versus men. This result is different from dr. Leli Saptawati et al in 2012 stated that TB lung infection is more likely to be suffered by men
than women. This is partly due to smoking habits that can increase the risk of pulmonary TB infection by 2.2 times (Zainul, 2009). Smoking habits will damage the lung defense mechanism called mucociliary clearance. Vibrating hairs and other materials in the lungs are not easy to remove infected infections due to vibration and other equipment in the lungs damaged by cigarette smoke. In addition, cigarette smoke increases airway resistance and causes easy leaking of blood vessels in the lungs, will also damage the macrophages that are cells that can phagocytes pathogenic bacteria (Widyasari et al, 2012). Smoking can disrupt the effectiveness of some defense mechanisms respiration. The results of cigarette smoke can stimulate the formation of mucus and decrease cilia movement. Thus, mucosal accumulation and increased risk of bacterial growth, including lung TB, may result in infection (Pertiwi, et al., 2012).
In the results of the study, it was found that patients with positive TB in men and women often occur in the age group above 54 years. The results of this study in accordance with the data riskesdas 2007 obtained in the prevalence of pulmonary tuberculosis in North Sulawesi Province tends to increase with age and the highest prevalence at age more than 65 tahun. In this study inversely related to his research Rikha Nurul Pertiwi et alus Faculty of Public Health UNDIP 2011 statistic analysis showed that age of respondent had no significant correlation with incidence of pulmonary tuberculosis and age 15-55 years old, had risk of developing lung tuberculosis with risk 0.667 times greater than age> 55 years (Lumentut, 2008). This is because the body's resistance began to decline after age 45 years so vulnerable to disease (Susanto AD, 2010)
The result of BTA examination which consisted of 68 sputum samples came from Bojonggede Public Health Center, which resulted in smear positive smear number of 14 people, consist of 8 women and 6 men. It turned out that the result of BTA is small, this is due to the research of many sputum that there is saliva (saliva) compared to sputum. This result is not in accordance with research Hendra Wijaya at the Faculty of Medicine, University of Sam Ratulangi in 2011 which states that the most positive smear the highest number of patients suspect tuberculosis there are 670 samples compared to negative BTA only 220 samples.

B. Sputum Inspection Result With PCR and BTA Technique

Based on PCR test using 19 sputum samples from Bojonggede Puskesmas, positive PCR test result was 11 samples and the positive percentage was 58%, while negative PCR test result was 8 samples and negative percentage was 42%. According to Diana Krisanti J (2005) stated that the examination with PCR technique compared with AFB and
Table 1. Comparison of Sputum Inspection Result with BTA and PCR Test

<table>
<thead>
<tr>
<th>NO</th>
<th>Patient’s Name</th>
<th>Age (year)</th>
<th>BTA test</th>
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cultures have a sensitivity of 30%, specificity of 80% and accuracy of 47%. Furthermore, the significance test with Mc Nemar gives a significant difference ($p < 0.01$). When compared with Tuberculosis bacterial culture method, Mycobacterium tuberculosis detection with PCR technique has 65% sensitivity, 40% specificity and 57% accuracy. Mc Nemar’s Meaning Test gave no significant difference (Diana K, 2005)

Compared with microscopic examination of BTA Mycobacterium tuberculosis detection with Tuberculosis bacterial culture method has 31.6% sensitivity, 81.8% specificity, and 50% accuracy. The significance test with Mc Nemar resulted in a significant difference ($p < 0.01$). Thus it can be concluded that detection of Mycobacterium tuberculosis by PCR technique is as good as bacterial tuberculosis culture (Diana K, 2005)
Based on the results of Andiny and Meizy (2012) study from all 36 sputum samples of TB lung tuberculosis patients negative from 25 men and 11 women, age 17-78 years obtained positive PCR test results were 13 samples (36.11%) and negative PCR examination results 23 samples (63.88%). PCR examination can be used to support the diagnosis if clinical, radiological support for lung TB with microscopic sputum smear negative, so no need to wait for the results of culture for two months. PCR is also a very useful, quick and effective examination for monitoring anti-TB treatment in patients as well as confirmation free certainty of M. tuberculosis. The weakness of the PCR test is that it can give positive results even though there are only 1-10 germs in the examination specimens or dead / dormant germs and contamination from other specimens. However, in this study, preventive measures have been taken to avoid false positives such as structuring.
Figure 8. The results of the PCR test from Tuberculosis patients consisted of 6 patients, namely: 14 = Mly - 15 = Wnr -, 16 = Ars +, 19 = Rk -, 20 = Hry +, 21 = Elz - , 22 = Gsr +

and sterilization of the room, discipline in work procedures, selection and sterilization of tools / materials. The results of the study were Basundari et al (2012), 70 sputum samples taken from suspect tuberculosis patients, examined using 3 types of examination: microscopic acid-resistant bacteria (BTA), PCR test and culture method that serves as gold standard (gold standard). The validity of the diagnosis is determined by calculating the sensitivity, specificity, positive and negative predictive value, the ratio of positive and negative tendencies, the accuracy of each? (Microscopic BTA and PCR). The sensitivity and specificity of microscopic examination of BTA was 77.2% (CI = 95%; 0.7837 - 0.7603) and 95.8% (CI = 95%; 0.96361 - 0.9523) with positive and negative predictive value of 89.4% and 90.1% with trend ratio (LR +) = 18.8 and (LR -) = 0.23. PCR test results showed 90% sensitivity and specificity of the examination (CI = 95%; 0.90705 - 0.89295) and 79% (CI = 95%; 0.7995 - 0.78043) with positive and negative predictive value 66% and 95% with trend ratio (LR +) = 3.18 and (LR -) = 0.11. As a diagnostic tool for pulmonary tuberculosis, PCR can validly distinguish patients with pulmonary TB and not pulmonary TB patients, but less reliable than the results of microscopic examination of BTA. (Basundari et al., 2002).
According to Kox (1996) Advantages of PCR include: The speed of examination is similar to microscopic, can still be for species identification, for early diagnosis, for the difficult diagnosis of Mycobacterium: to identify Mycobacterium in samples such as urine, skin or other tissues, cerebrospinal fluid in which the amount of Mycobacterium is very low (although there is 1 Mycobacterium in the preparation), another advantage is that PCR can be used as a confirmation of treatment outcomes.

Although the sensitivity of PCR diagnosis is higher than that of BTA, but the number of false positive PCR is greater. Kox states that the problem in PCR technique is a false error in the PCR technique of false positives, this is due to contamination at the amplification stage in the PCR probe process. Another drawback is that PCR checks are very expensive.

The results of PCR use in the laboratory have been reported to show that PCR is not reliable as a field diagnostic device, but it is more reliably used in laboratories with high facilities with adequate equipment and performed by experienced technicians, according to the results of the study this. From the results of this study it can be argued that the use of PCR in the diagnosis of TB is not necessary if on positive microscopic examination deangan clinical picture of patients who support towards tuberculosis infection, especially in endemic areas. The diagnosis of PCR is indispensable in special circumstances in which the results of microscopic examination are negative but clinical features indicate the direction of pulmonary tuberculoid infection or on suspected tuberculosis cases of the pulmonum (Kox, 1996).

C. Limitations of Research

1. Time for research is very limited, because it is done between teaching and learning activities and practicum.

2. Sampling can only be done on Wednesday and Friday at around 11:00, so until the laboratory is late afternoon.

3. To grow bacterial culture this takes a long time about 8 - 14 weeks.

CONCLUSION
1. BTA stain result from Bojonggede Puskesmas was 68 samples, BTA positive was 13 samples and the percentage of BTA positive was 19%, while BTA negative was 55 and the percentage was 81%.

2. Comparison of PCR test results with staining Bhi Zhiel Neelsen showed PCR test results a positive percentage greater than smear stain.