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Comparison of Different DNA Extraction Methods for the Diagnostic of *Mycobacterium tuberculosis* using Real-Time PCR Technique

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Abstract: ***During the study period (July to December 2016) a total of 188 samples (sputum and blood from each person) have been tested in the National Reference Laboratory (NRL) at the Institute of Chest and Respiratory Diseases / Baghdad. The calculation of sensitivity, specificity, positive predictive value and negative predictive value for any procedure used for detection will depend on the result of the standard procedure which is culture method , so we consider real positive case if it is positive in culture method and the same situation for the negative case. Three methods were submitted for DNA extraction, two from sputum and another one from blood. A Realline Kit and MTB Robogene kit successfully extracted DNA from Mycobacterium within all sputum samples. MTB Blood kit successfully extracted DNA from Mycobacterium within all blood samples A few general instructions about DNA extraction from microorganisms can be made. The range of DNA concentrations using Realline Kit, MTB Robogene kit and MTB blood kit were (1.65-14.43) ng/µl, (1.34- 13.23) ng/µl and (1.2-11.76) ng/µl respectively. The mean concentration for the previously three mentioned kit were 9.7 ng/µl, 7.3 ng/µl and 3.2 ng/µl. Detection of M. tuberculosis by real time PCR using Realline kit showed that among the total 188 specimens (Suspected Group), 112 (59.57%) of the specimens were positive and 76 (40.4%) negative which detected by Real-Time PCR using Realline Kit. All these samples are sputum. This study showed the percentage of sensitivity, specificity, PPV and NPV of Realline kit were 92.43%, 97.1%, 98.21% and 88.15 % respectively. Detection of M. tuberculosis by real time PCR using MTB Robogene kit showed that among the total 188 specimens (Suspected Group), 111 (59.04%) of the specimens were positive, and 77 (40.95%) negative specimens by using MTB Robogene kit, the samples are sputum. the sensitivity, specificity, PPV and NPV of our study were 87.3 %, 89.85%, 93.69% and 80.51% respectively. Detection of M. tuberculosis by real time PCR using MTB- DNA Blood showed that among total 188 specimens (Suspected Group), 59 (31.38%) were positive in PCR and 129 (68.61%)were negative, DNA was extracted directly from blood and then tested in MTB Robogene kit. The present showed the percentage of the sensitivity, specificity, PPV and NPV to be 47.89%, 97.1%, 96.6% and 51.93.***

Keywords: *Mycobacterium tuberculosis*, DNA extraction, sensitivity, specificity, Real-Time PCR

1. Introduction

TB is considered to be one of the deadliest bacterial infections in the world infection with this disease can be fatal and it had a large impact on global health getting international attention with increasing number of cases worldwide developed and developing countries were critically included (WHO 2015). MTB is the main causative agent of TB which is an aerobic pathogenic bacillus that establishes infection in the lungs its believed that two billion people are carrying non-eradicated intra-granulomatous Tuberculosis bacilli as LTBI (short for latent tuberculosis infection) and around 10% of those people will be infected with active tuberculosis during their lifetime (Gerald et al., 2010). The MTB complex consists of: *M tuberculosis M canetti M microti M pinipedi M africanum M bovis and M caprae* most of infections in humans are caused by *M tuberculosis* with a small number of infections caused by *M africanum M bovis* (LoBue *et al.*, 2010). It is estimated that 1/3 of the population (around 2 billion people) are infected with TB bacilli however only 5-10% of people get infected with the active disease (WHO 2007) the remaining 90% will initially be experience no symptoms of the disease and will experience latent disease which reactivation may happen the world health organization estimate that in 2008 there were 9.4 million cases of TB lead to 1.3 million deaths (WHO 2009).The incidence infections and disease varies greatly geography wise as the estimated number of cases in 2008 occurred in Asia (55%) and Africa (30%) (WHO 2009). Iraq is one of the 7 countries of the Region with a high infection rate of TB and has 3% of total number of cases worldwide there are and estimated number of 20000 TB patients in Iraq and more than 4000 estimated deaths due to TB (WHO 2014). In Iraq the disease is an epidemic, Iraq is one of the countries in WHO-EMR (WHO Eastern Mediterranean Region) with a pretty high infection rate about 56 cases per 100, 000 people every year for all forms of TB which have a low detection rate around (45%) (Merza *et al.*, 2011; Al-Khafaji *et al.*, 2014). Around 3000 people die from this disease every year which is spread by coughing and sneezing (Ali et al., 2013).Every second s new person is infected and every 15 seconds a new one dies to TB infections development of MDR-TB (short for multidrug-resistant tuberculosis) and XDR-TB (short for extensively drug-resistant tuberculosis) strains together with the spread of risk factor such as HIV (human immunodeficiency virus) AIDS (acquired immunodeficiency syndrome) and diabetes (Corbett et al. 2003, Resterpo *et al.*2008)strengthens the

urge to develop treatment for infection strategies and continue to make MTB a health concern in developed countries. In 2012 new cases of TB were around 8.6 million and 14 million worldwide and around 1.3 million deaths due to the disease (WHO 2013) this is equivalent to every 100, 000 people there is around 122 people infected with TB most of the cases were in Asia (58%) and Africa (27%). Tuberculosis is a disease with protean manifestations. The clinical presentation of TB can mimic several diseases and can be a diagnostic problem even in endemic areas. Virulence and load of the infecting *Mycobacterium*, the immune status of the host, the organ system involved, all influences the clinical manifestations of tuberculosis. Tuberculosis is diagnosed by finding *Mycobacterium tuberculosis* bacteria in a clinical specimen taken from the patient. While other investigations may strongly suggest tuberculosis as the diagnosis, they cannot confirm it. A complete medical evaluation for tuberculosis must include a medical history, a physical examination, a chest X-ray and microbiological examination (of sputum or some other appropriate sample). It may also include a tuberculin skin test and surgical biopsy (Ismael and Ray, 2004).Smear microscopy is the most widely used test for the diagnosis of tuberculosis (TB). The majority of laboratories use conventional light microscopy to examine Ziehl-Neelsen stained direct smears, documented to be highly specific in areas with a high prevalence of TB but with varying sensitivity (20-80%) (WHO, 2009).A definitive diagnosis of tuberculosis can be made by culturing Mycobacterium tuberculosis organisms from a patient specimen most often sputum, but may also include pus, CSF, biopsied tissue etc. (Kumar et al., 2007). Many types of cultures are available. Traditionally, cultures have used the Lowenstein-Jensen (LJ) and Middlebrook media (7H9, 7H10, and 7H11) (Drobniewski *et al.*, 2013). In order to overcome conventional methods low sensitivity and diagnostic delays, nucleic acid amplification (NAA) tests have been introduced. The Xpert MTB/RIF is a cartridge based, automated diagnostic test that can identify *Mycobacterium tuberculosis* (MTB) and resistance to rifampicin (RIF). It was co-developed by Cepheid, Inc. and Foundation for Innovative New Diagnostics, with additional financial support from the US National Institutes of Health (NIH) and technical support from the University of Medicine and Dentistry of New Jersey (UMDNJ). In December 2010, the World Health Organization (WHO) endorsed the Xpert MTB/RIF for use in TB endemic countries (WHO, 2010d) and declared it a major milestone for global TB diagnosis. This followed 18 months of rigorous assessment of its field effectiveness in TB, MDR-TB and TB/HIV co-infection (Small and Pai, 2010). This test, and others that are likely to follow, have the potential to revolutionize the diagnosis of TB (Small and Pai, 2010; Van Rie *et al.*, 2010). Real time PCR combines fluorescent probe detection with PCR chemistry in the same reaction vessel in general both amplified product detection and PCR are considerably faster than conventional PSR and other detection methods the both also an hour or less to complete (Espy et al., 2006).There is several fluorescence formats available for correlating the amount of PCR product with fluorescence signals that are: Minor groove binder (MGB) DNA probes, SYBR Green I dye, TaqMan probes, Hybridization probes, Scorpions, and Molecular beacons (Parashar *et al.*, 2006). Microbiological diagnostics is being revolutionized by real-time PCR because of the sensitivity and specificity of detection for determination of variants the sensitivity of real time PCR in clinical specimens ranges from 71.6%- 98.1% more than conventional staining (46.3%) and culture techniques (41.8%) and it has a 100% mycobacteria detection specificity (Parashar *et al.*, 2006).Unlike traditional culture methods which focus on measuring live pathogens real time PCR can measure both live and dead pathogens (Hein et al., 2001).

2. Materials and Methods

2.1 Patients and Sample Collection

The study was conducted at National Reference Lab (NRL) of tuberculosis/Baghdad**,** total of 188 patient suspected and 80 normal people. Two samples have been gathered from every patient. first, one became taken from patient when he just reached the institute; second pattern accrued at early morning earlier than breakfast, the early morning collection represents the pulmonary secretions accrued in a single day, and consequently it typically has a better positivity. Gathered specimens were stored at –20°c until use (IUATLD, 2000; ssengooba *et al.*, 2012). ziehl-neelsen stain sputum smears were tested for the presence of pulmonary acid fast bacilli. also forty specimens from healthful men and women have been accumulated as negative control**.**

2.2 DNA extraction using spin column (innuPREP Mycobacteria DNA Kit)

Transfer 200 µl of sputum into 1.5 ml reaction tube (DNA_D1 which already supplied with the amplification kit MTB DNA Qualitative kit, containing Internal control DNA) and add 200 µl NAC buffer. Vortex shortly and incubate the sample at room temperature under continuous shaking for 20 minutes. Centrifuge the sample at 10000 rpm for 15 minutes. Remove the supernatant carefully, but completely. Add 200 µl TE buffer to the bacterial pellet and re-suspend the pellet completely**.** Add 15 µl Lysozym (stock solution 10 mg / ml in TE buffer). Mix by pulsed vortexing for 5 s. Incubate at 37°C for 30 minutes. After lysis with lysozyme add 200 µl lysis solution TLS and incubate the tube at 95 °C for 20 minutes. After incubation at 95 °C place the tube on ice for 2 minutes. Open the tube and add 25 ul Proteinase K and incubate at 50°C for 30 minutes. Apply 600 µl of the sample to the Spin Filter (Blue) located in a 2 ml receiver tube. Close the cap and centrifuge 12000 rpm for 2 minutes. Discard the receiver tube with the µfiltrate. Place the spin filter into new 2 ml receiver tube. Open the spin filter and add 500 µl washing solution HS, close the cap and centrifuge 12000 rpm for 1 minutes. Discard the receiver tube with the filtrate. Place the spin filter into new 2 ml receiver tube. Open the spin filter and add 650 µl washing solution MS, close the cap and centrifuge 12000 rpm for 1 minutes. Discard the receiver tube with the filtrate. Place the spin filter into new 2 ml receiver tube. Centrifuge at max. Speed for 2 minutes to remove all traces of ethanol. Discard the 2 ml receiver tube. Place the Spin Filter into 1.5 ml Elution Tube. Carefully open the cap of the spin filter and add 50 µl pre-heated

Elution Buffer. Incubate at room temperature for 2 minutes. Centrifuge at 8000 rpm for 1 minutes.

2.3 DNA extraction using magnetic beads (RealLine Mycobacterium tuberculosis)

Prior to use, take the kit out of the refrigerator and keep the analyzed samples and reagents from the kit at (18 - 25) °C for 30 minutes. Add 1 ml of Recovery Solution for Control samples (RSC) into a vial with Internal Control (IC) sample, mix gently, keep for 15 minutes, and then carefully mix once again. IC should be stored at (2 - 8) °C and used within 1 month of preparation. Negative Control (NC) sample is ready to use. Once opened, negative control samples should be stored at (2 - 8) °C and used within 1 month. Prior to use, warm up Lysis Solution at (50 - 60) °C and mix thoroughly to dissolve the precipitated material. Vortex Sorbent to a condition of homogeneous suspension. Add 80 µl of Sorbent suspension into a vial with Lysis Solution. Mix carefully Collect sputum in clean collection cup with leak-proof lid. Add equal volume of Solution for sputum preparation, mix the sample thoroughly and keep at room temperature for 10 min. Use 100 µl suspension for nucleic acids isolation. Do not capture clumps of mucus. Determine the appropriate number of reaction tubes needed for patient specimen and

Control testing. Label each 2.0 ml tube for each patient specimen and control sample. Add 30 µl of IC to each tube. Add 100µl of NC to the tube labeled NC Add 30 µl of PC (Positive Control) and 70µl to the tube labeled PC. Add 100 µl of corresponding sample of patient specimen to the appropriately labeled tube using a separate tip with filter. Add 300µl of Lysis Reagent with Sorbent to each tube. Vortex for 5 - 10 seconds. Place the tubes into Thermo Shaker, and incubate for 10 minutes at 65 °C and 1300 rpm. Spin shortly to collect the drops. Add 400 µl of Solution for DNA/RNA Precipitation in each tube. Vortex for 10 - 15 seconds. Centrifuge at 13000 rpm for 5 minutes at room temperature. Trying not to shake up a pellet, place the tubes to Magnetic Rack. Using a new tip for each sample, carefully remove the supernatant without disturbing the pellet. Add 500 µl of Wash Solution in each tube. Vortex vigorously for 10 - 15 seconds. Centrifuge at 13000 rpm for 2 minutes. Trying not to shake up a pellet, place the tubes to Magnetic Rack. Using a new tip for each sample, carefully remove the supernatant without disturbing the pellet. Add 300 µl or Wash Solution to each tube. Vortex vigorously for 10-15 seconds. Centrifuge at 13000 rpm for 2 minutes. Trying not to shake up a pellet, place the tubes to Magnetic Rack. Using a new tip for each sample, carefully remove the supernatant without disturbing the pellet. Dry the pellet with open caps for 2-3 minutes at room temperature (18- 25)°C. Add 200 µl (up to 600 µl, if necessary) of Specimen Diluent to each tube. Vortex vigorously for 10 -15 seconds. Place the tubes into Thermo Shaker, and incubate for 5 minutes at 65 °C and 1300 rpm. Then centrifuge for 1 minute at 13000 rpm.

2.4 DNA extraction using MTB- DNA Blood

Pipette 1ml sample into a sample tube (ST tube), then add 400 ul buffer and vortex at 14000 rpm for 15 s to mix, let stand on the benth at room temperature for 5 minutes. Buffer CM is a chaotropic buffer that lyses the human cells. For optimal results it is important to mix thoroughly. Briefly centrifuge to clear the lid, add 400 ul buffer DB1 and 10 µl MolDNase B to the lysate and immediately vortex for 15 s. Let stand on the bench at room temperature for 15 minutes. Centrifuge the ST, carefully remove the supernatant by pipetting and discard. Add 1 ml buffer RS and vortex to resuspend the sediment. Centrifuge ST tube for 5 minutes carefully remove the supernatant by pipetting and discard. Add 80 µl buffer RL to the ST tube resuspend the sediment by vigorous vortexing, briefly centrifuge to clear the lid. Add 20 µl BugLysis solution, vortex for 15 s and incubate tube in a thermomixer at 37 °C and 1000 rpm for 30 min. Adjust the temperature of themomixer to 56°C, briefly centrifuge the ST tube. Add 150 µl buffer RP and 20 ul Proteinase K to the tube. Vortex at full speed for 15 s and incubate at 56 °C and 1000 rpm for 10 min. Thereafter, adjust the temperature of the thermomixer to 70 °C (make sure that the vial with buffer ES is placed in the mixer) Briefly centrifuge the tube to remove from the lid. Add 250 µl buffer CS and vortex at full speed for 15 s. Briefly centrifuge the tube and add 250 µl binding buffer AB and vortex at full speed for 15 s. Briefly centrifuge and transfer the lysate to a spin column (SC), close lid and centrifuge loaded column 14000 rpm for 30s, open the lid, remove the spin column, discard the collection tube with flow –through and replace the column into a new collection tube (CT). Add 400 µl buffer WB to the spin column, close lid and centrifuge at 14000 rpm for 30 s, open the lid, remove the spin column, discard the collection tube with flow –through and replace the column into a new collection tube (CT). Wash the spin column with 400ul buffer WS by centrifugation at 14000 rpm for 3 minutes. Carefully remove the column from the centrifuge, avoid splashing of the flow –through to the column, transfer column to a 1.5 ml Elution tube (ET), discard the collection tube containing the flow – through. Place 100 µl buffer ES (tube in the thrmomixer is preheated to 70°C) to the centre of the column, close the lid and incubate for 1 minutes at room temperature, centrifuge at 14000 rpm to elute the DNA.

2.5 Detection of TB using RoboGene® MTB DNA Qualitative Kit

Centrifuge the MTB_D4 briefly at full speed to collect the lyophilized Reagent Mix on the bottom of the tube. Add 200 µl PCR grade water DNA to MTB_D4; close the tube, mix by brief vortexing followed by brief centrifugation at full speed. Incubate at 37°C for 20 min using a thermal mixer (800 -1, 000 rpm), mix by brief vortexing followed by brief centrifugation at full speed**.** Dissolved reagent mix can be stored at 2-8 °C and always protected from light up to 14 days. Prepare the 1x Master Mix according to the following table. Mix by vortexing for at least 3 s followed by brief centrifugation. Identify sample tubes (MTB_D2_xx) and standards (MTB_D3_xx) carefully and place them onto a suitable rack. Add 20 µl 1x Master Mix to sample tubes and each tube with standards. Add 5 µl PCR grade water to tubes that serve as NTC and to all quantification standards containing the 1x Master Mix. Do not exceed a final reaction volume of 25 µl Add 5 µl of eluate from DNA isolation to the respective sample tubes containing the 1x Master Mix. Do not exceed a final

reaction volume of 25 µl. Cover the tubes with optical tape (OT_AB) according to the required. Size and cover sample and quantitation standard strips carefully. Use of an appropriate applicator for fixing the tape at the tube surface of the strips is recommended. Centrifuge reaction plate with tubes at 200x g for 1 min. Program the applied real-time PCR platforms as indicated in below Table (2. 1) and start the program.

Table 2.1: Cycling conditions for RoboGene® MTB DNA Qualitative Kit

Stages	*Temperature*	*Time*	*No. of cycles*
Stage 1	95°C	10 min.	1 cycle
Stage 2	95°C	30 sec	45 cycle
	59°C*	1:50 min	

3. Results and Discussion

3.1 Population Studies

The data set in Table (3.1), exhibited the percentage of infected patients were male 73 constituted (70.87) to female 33 that constituted (32.03 %), with over all male to female ratio of 2.21 (73/33) with a highly significant difference (P≤0.01). These results were in accordance with previous results reported by Shaker, (2013) and Sabah, (2015). The attributable for higher men incidence are poorly understood, and require additional research to identify correlating hazard agents. Difference between male and female susceptibility to TB may result partly from biological differences (i.e. sex differences), cultural and the economic state of the society cause to gender differences having access to health care. Various studies introduced evidence for a possible role of the X chromosome and sex hormones (i.e. testosterone) in susceptibility to TB (Neyrolles and Quintana-Murci 2009; Bayingana et al., 2014; Boum et al. 2014).Hormones related with sex may be a compelling factor for this difference, testosterone for example decreases the macrophage activation process as well as pro-inflammatory cytokines production, on other hand estrogens are pro inflammatory mediator's inducer. Recent study mentions that this difference may be due to more existence of men in the community and more an unexpected meeting with carriers and the disease's risk factors such as cigarettes and narcotic materials (Bini EI *et al.*, 2014) (Babamahmoodi et al., 2015).

Table 3.1: Distribution of TB patients according to their gender

Gender	Healthy n (%) AFB –VE	Suspected n, +ve n (%) AFB +ve	Suspected n, - ve n (%) AFB +ve	Total n, +ve n (%) AFB +ve	Total n, -ve n (%) AFB –ve
Male	42 (100%)	110, 73 (38.8%)	110, 37 (33.63)	152, 73 (48.02)	152, 79 (51.97)
Female	38 (100%)	78, 30 (15.9%)	78, 48 (61.53)	116, 30 (25.28)	116, 86 (74.13)

3.2 Extraction and Concentration of DNA

268 samples which were suspected and healthy persons underwent to deoxyribonucleic acid (DNA) extraction. Three methods were submitted for DNA extraction, two from sputum and another one from blood. Realline Kit and MTB Robogene kit were successfully extracted DNA from *Mycobacterium* within all sputum samples. MTB Blood kit was successfully extracted DNA from *Mycobacterium* within all blood samples A few general instructions about DNA extraction from microorganisms can be made. The thick cell wall of gram positive bacteria (i.e. *Mycobacterium*) is more difficult to damage than the relatively thinner cell wall of gram negative bacteria. Materials that may prevent amplification should be removed. The released nucleic acids must be preserved in an aqueous solution in order to avoid degradation. Extracted DNA should be eluted into a small volume so as to maximize detection. The range of DNA concentrations using Realline Kit, MTB Robogene kit and MTB blood kit were (1.65-14.43) ng/µl, (1.34- 13.23) ng/µl and (1.2-11.76) ng/µl respectively, The mean concentration for the previously three mentioned kit were 9.7 ng/µl, 7.3 ng/µl and 3.2 ng/µl these values is somewhat similar to Sabah (2015) with range of DNA concentrations (1.24-12) ng/µl and mean concentration 3.7 ng/µl. (Table 3.2) All these different concentrations of DNA were enough to successful DNA amplification when detected by real time PCR. These result were accepted in a study done by AL-Noomani *et al.*, (2010) (Figure 3.1, 3.2, 3.3)

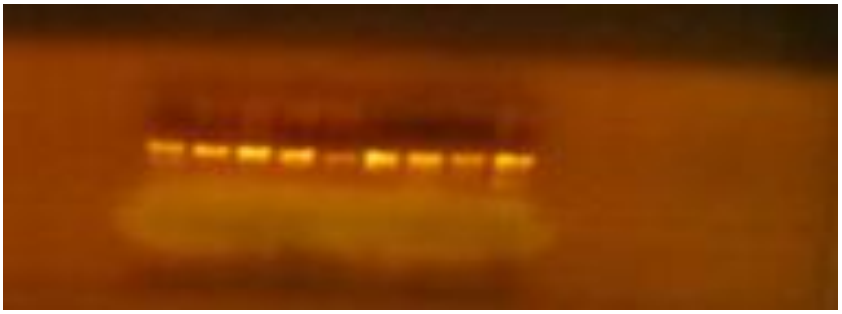

Figure 3.1: Genomic DNA electrophoris extracted by Realline Kit

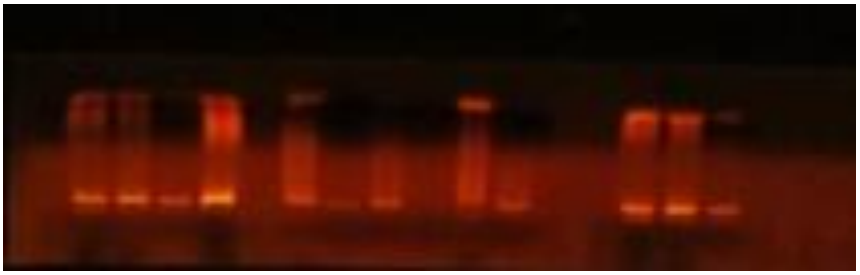

Figure 3.2: Genomic DNA electrophoris extracted by MTB Kit

Figure 3.3: Genomic DNA electrophoris extracted by MTB blood Kit

Table 3.2: DNA extraction method related with concentration and purity

DNA extraction method	Range of DNA concentration ng/µl	Mean of DNA concentration ng/µl	DNA Purity 260/280 mean	260/230 Mean
Realline TB	1.65-14.43	9.7	1.92	1.43
MTB Robogene	1.34- 13.23	7.3	1.84	1.12
MTB blood	1.2-11.76	3.21	1.78	1.01

3.3 Detection of *M. tuberculosis* by real time PCR using MTB Robogene kit

The results revealed among total 188 specimens (Suspected Group), 111 (59.04%) of the specimens were positive, and 77 (40.95%) negative specimens by using MTB Robogene kit. (Table 3.12) (Figure 3.13), the sensitivity, specificity, PPV and NPV of our study were 87.3 %, 89.85%, 93.69% and 80.51% respectively, the sensitivity is greater than value found by Eddin and Fawza (2012) which was 66.6% as in our study, DNA extracted from sputum while in Eddin study, DNA extracted from blood. There are a high percentage of PCR inhibitor that exist in the blood sample type specifically that came from Human genome, so we should to exclude the human genome then start the extraction procedure for Bacterial DNA in the blood (Handschur *et al.*, 2009). Real-time PCR was negative in 15/119 TB cases (12.6%); this reduction of the sensitivity might be due to the uneven distribution of bacilli in the sample (Tortoli2007). The suboptimal extraction of nucleic acids was addressed in our study by the detection of human beta-globin DNA in all extraction products. From non-TB patients, real-time PCR was positive in 7/69 cases (10.14%), and this false positivity may due to laboratory contamination (Ani, A. *et al.*, 2009)

Table 3.12: Distribution of TB patients according to their gender using MTB Robogene kit in Real-Time PCR technique

Gender	Healthy n (%) RealLine TB kit	Suspected n, +ve n (%)MTB Robogene kit	Suspected n, -ve n (%)MTB Robogene kit	Total n, +ve n (%)MTB Robogene kit	Total n, -ve n (%) MTB Robogene kit
Male	42 (100%)	110, 76 (69.09)	110, 34, (30.9)	152, 76 (50)	152, 76 (50)
Female	38 (100%)	78, 35 (44.87)	78, 43, (55.12)	116, 35 (30.17)	116, 81 (69.8)

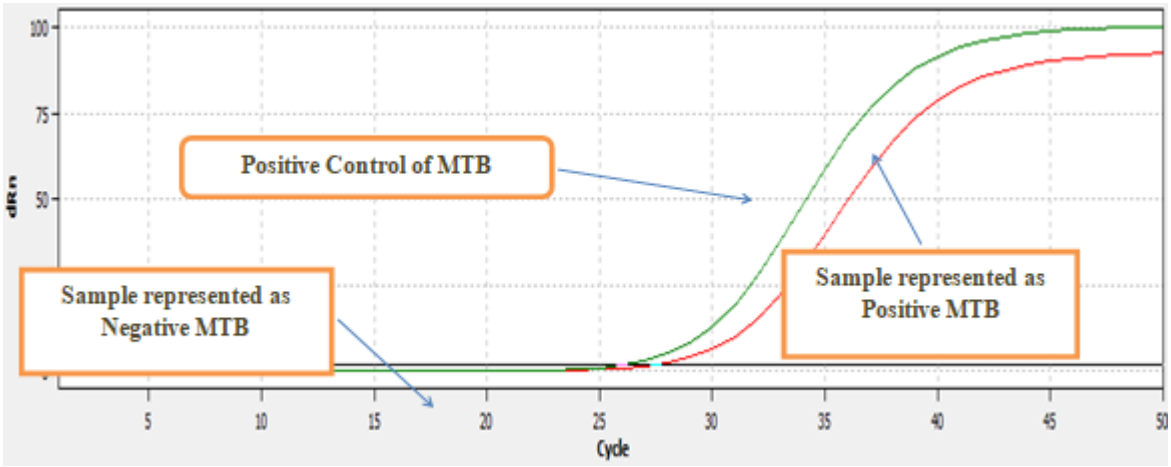

Figure 3.13: Positive and negative case detected by MTB Robogene Kit

Table 3.13: The percentage of sensitivity, specificity, PPV and NPV of MTB Robogene in comparing with sputum culture

MTB Robogene kit	L.J culture +ve	L.J culture –ve	Total	Sensitivty (%)	Specificity (%)	PPV (%)	NPV (%)
MTB Robogene kit +	104	7	111	87.3	89.85	93.69	80.51
MTB Robogene kit -	15	62	77				
total	119	69	188				

3.4 Detection of *M. tuberculosis* by real time PCR using MTB- DNA Blood

The results revealed among total 188 specimens (Suspected Group), 59 (31.38%) were positive in PCR and 129 (68.61%) were negative; DNA was extracted directly from blood and then tested in MTB Robogene kit. (Table 3.14) (Figure 3.9) showed the positive cases. Our study showed the percentage of the sensitivity, specificity, PPV and NPV were 47.89%, 97.1%, 96.6% and 51.93. The sensitivity value in our study is somewhat greater than the sensitivity found by Bwanga et al., (2015) which was 33% as he extracted DNA from 1 ml blood (Study A), while the sensitivity is increased with noticeable value to 71% when DNA extracted from 9 ml blood (Bwanga *et al.*, 2015). The sensitivity of PCR is represented in direct way relation with the real number of colony forming units that represent in blood sample (Baker S 2010). The extraction of DNA from a high volume starting blood will improve the performance of PCR technique, on another hand the remaining of human DNA beside bacterial DNA will induce false-positive PCR signals because of the binding of the non-specific primers and the consequence of these results (false-negative) definitely decrease the percentage of sensitivity.

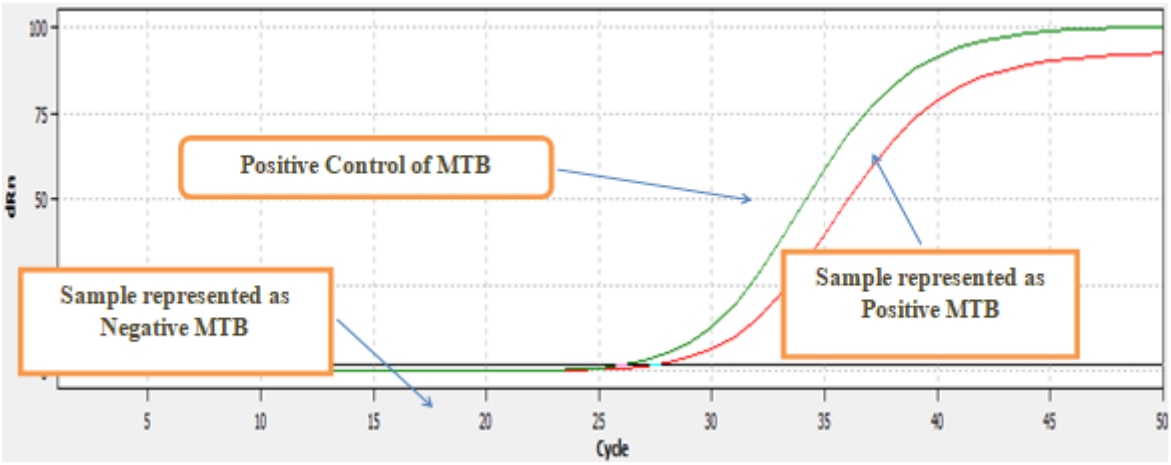

Figure 3.14: Positive cases of blood samples using MTB blood kit in DNA extraction

Table 3.14: Distribution of TB patients according to their gender using MTB- DNA Blood in PCR

Gender	Healthy n (%) MTB- DNA Blood kit	Suspected n, +ve n (%) MTB- DNA Blood kit	Suspected n, -ve n (%)MTB- DNA Blood kit	Total n, +ve n (%)MTB- DNA Blood kit	Total n, -ve n (%) MTB- DNA Blood kit
Male	42 (100%)	110, 42 (38.18)	110, 68, (61.81)	152, 42 (27.63)	152, 110 (72.36)
Female	38 (100%)	78, 17 (21.7)	78, 61, (78.2)	116, 17 (14.65)	116, 99 (85.34)

Table 3.15: The percentage of sensitivity, specificity, PPV and NPV of MTB- DNA Blood in comparing with sputum culture

MTB- DNA Blood kit	L.J culture +ve	L.J culture –ve	total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
MTB- DNA Blood kit +	57	2	59	47.89	97.1	96.6	51.93
MTB- DNA Blood kit-	62	67	129				
Total	119	69	188				

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