International Journal on Emerging Technologies 11(3): 182-187(2020)

ISSN No. (Print): 0975-8364
ISSN No. (Online): 2249-3255

Development and Validation of Indigenously developed DNA Detection System for *M. tuberculosis*

Yogesh Patil[1] and Mansee Thakur[2]
[1]Ph.D. Scholar, Department of Medical Biotechnology,
MGMCRL & MGMSBS, MGMIHS, Navi Mumbai, India.
[2]Professor and HOD, Department of Medical Biotechnology,
MGMCRL & MGMSBS, MGMIHS, Navi Mumbai, India.

(Corresponding author: Mansee Thakur)
(Received 28 January 2020, Revised 01 April 2020, Accepted 03 April 2020)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: India ranks number one in terms of incidence and prevalence rates for Tuberculosis across the globe and it is a major healthcare problem faced by government. Lot of efforts had been taken to control the spread of the Tuberculosis, still the rates are uncontrolled and every year incidence and prevalence rates are increasing. The major cause of spread of this disease is undiagnosed and untreated patients, due to lack of India specific healthcare facilities at primary healthcare centers. Conventional methods are restricted to tertiary healthcare setups due to high cost and specific operating conditions. Present study addresses evaluation of India specific indigenous and portable TB detection system and conducted from June 2019 to December 2019, in Central Research Laboratory (CRL), MGM Medical College and Hospital, Kamothe, Navi Mumbai, India. The sensitivity and specificity of the developed system was compared with conventional TB diagnostic tools such as Real Time PCR and Smear Microscopy technique. In the present study the sensitivity and specificity of DNA Detection system was found to be 100% and 97% as compared to 100% sensitivity as well as specificity of Real Time PCR assay and 63.16% sensitivity and 100% specificity of most commonly used smear microscopy technique. The developed device can be suitable for screening of infectious diseases at rural setup with conventional PCR in time bound and cost effective manner.

Keywords: Real Time PCR, Smear Microscopy, *Tuberculosis*, Conventional PCR, Indigenous DNA detector system.

I. INTRODUCTION

Tuberculosis (TB) is a big global public health epidemic with ~9.6 million new infections and 1.5 million deaths in 2014 [1]. The rates of incidence are increasing from past 20 years and reaching to new heights every year and now Tuberculosis is one of the first cause of mortality in the world in terms of deaths due to infectious diseases [2]. Tuberculosis is still the major public health concern in India and shares about 30% of the world's tuberculosis patients [3]. Presently TB is primarily diagnosed by microscopic technique using AFB, which lacks adequate sensitivity [4-6]. The gold standard culture method takes several weeks. Detection of drug resistance takes even longer [7, 8]. The developing countries like India it becomes difficult for effective diagnosis of tuberculosis hence containment of disease is still challenging task [9]. Since delayed diagnosis enhances chances of further transmission; efforts are being made to reduce this time of detection. Development of molecular diagnostic methods seems to offer lot of promise in this context. These molecular methods are generally based on Nucleic acid Hybridization [10] or PCR. Both traditional as well as Real Time PCR [11]. The Hybridization based approach appears to reduce the time to about few days. In order to design and develop clinically sensitive and specific molecular tests it is essential to have profound understanding disease detection. So the molecular diagnostic based techniques could be more promising as these could reduce this time to few hours. One of the molecular techniques is Polymerase chain reaction (PCR) which has revolutionized the detection of nucleic acids. This method is the most commonly used laboratory technique for DNA amplification and is capable of detecting as little as a single copy of DNA or RNA.

It is a popular DNA amplification technique and can create millions of amplicons of a target sequence in a short period of time [12-15].

This technique has been widely utilized for a variety of applications, including the detection of pathogenic bacteria viral, fungal etc. detection simply because specific DNA sequences can serve as reliable bacterial biomarkers and the amplification power of PCR permits the detection of a small number of bacteria before they can grow to infectious quantities [16-19]. In spite of several advantages well adopted technique in clinical laboratories, it has not become commonly used point-of-care or field tools. One significant roadblock that prevents such applications that are very expensive as the cost associated with these equipment's are very high as well requires regular maintenance, uninterrupted electrical line power and cannot be afforded by primary healthcare centers. However, there have been significant efforts towards miniaturizing PCR machines [19-22]. Another significant barrier that restricts PCR from becoming a popular field tool is the lack of simple yet effective signal transduction mechanisms that permit detection of PCR products without the use of expensive equipment (such as real-time PCR machine products detection uses expensive CCD cameras and Detectors which overall increases the cost of the equipments and cost of the test per sample) and complicated process (such as DNA separation by gel electrophoresis).

There are number of efforts not only to develop molecular diagnostic test but try to adopt these test on lab on chip, portable devices. Development of such Device can not only reduce the cost but also useful for POC application. In India rural areas lack Infrastructure for diagnosis and treatment of disseminated TB. Hence development of such device can make paradigm shift in health management.

In addition, such device should offer higher sensitivity and specificity, this should be rapid, robust, compliant with decentralized use, inexpensive, have long shelf life, amenable to large-scale production, require minimal laboratory infrastructure and personnel training. This wish list has not yet been fulfilled, as no low-cost point-of-care (POC) test for TB exists. One approach to address this problem is to develop simple indigenous, portable and low cost DNA Detection system for endpoint detection of amplified product of PCR. The development of biosensors with novel attributes is now possible with the rapid developments in micro & nanotechnologies. Present invention addresses the Endpoint detection of PCR Products with the help of rudimentary battery operated laser based florescence Detector System case study using detection of *mycobacterium tuberculosis* as an example. Which are Affordable and portable, usable in rural settings, cost effective, Battery Operated and Operated by Semiskilled Operator.

II. MATERIALS AND METHODS

A cross-sectional laboratory-based study was conducted on 69suspected sputum samples from TB subjects visiting to outpatient department of MGM hospital, Kamothe, Navi Mumbai who were screened & clinically examined for tuberculosis. Aim to assess the sensitivity and specificity of indigenously developed DNA Detection system for M. Tuberculosis (TB) in comparison with smear microscopy by acid fast bacilli staining and MTB PCR by RT PCR assay. Samples were processed in under aseptic conditions inside the biosafety cabinet.

Specimen Preparation: Each sample was used for three procedures, one for decontamination processing and two (1 mL each) for DNA extraction and PCR. Samples were decontaminated, homogenized of sputum samples by NALC-NaOH Method was used for decontamination of collected samples [23].Two drops from concentrated and homogenized samples were used for indirect smear preparation. Smear preparation, ZN staining and slide reading were carried out according to the recommendations outlined in the Manual of Tuberculosis Bacteriology [24]. Samples containing 1 mL sputum were centrifuged at 1800g for 15 minutes; supernatants were discarded, and pellets were used for DNA isolation.

DNA extraction: DNA was extracted from the decontaminated samples by using commercially available HiPurA™ Mycobacterium tuberculosis DNA Purification Kit (Cat No.MB545) from Himedia with slight modification in primary incubation steps with lysozyme [25].

DNA Amplification: A PCR protocol was performed using Primers were manufactured specific for insertion sequence *IS6110*, sequence most frequently used to detect *Mycobacterium Tuberculosis* shown in Table 1.in final 25 µL reaction volume comprised of Hi-SYBr Master Mix (MBT074) by mixing 12.5 Micro liters of Sybergreen master mix, forward and reverse primers 1µl each, template DNAµl and PCR grade water was added to balance the reaction volume to 25 µl [26].

Table 1: Primers sequences specific for IS6110 insertion sequence of *Mycobacterium Tuberculosis.*

Primer Name	Sequence
IS6110-F	5' – CTCGTCCAGCGCCGCTTCGG – 3'
IS6110-R	5' - CCTGCGAGCGTAGGCGTCGG –3'

The reaction mixture is subjected to initial denaturation of 95 for 5 minutes followed by 35 cycles of 95 for 30 seconds, 58 for 60 seconds and 72 for 60 seconds followed by 5 min final extension step.

Detection by Real Time PCR (Sybergreen based Assay): Recently, real-time PCR has been widely used because of its excellence performance to overcome the limitation in conventional PCR method. The RT-PCR is believed to be more rapid than conventional PCR with excellent reproducibility that only requires less than one hour in diagnosing MTB [27] (after DNA extraction). According to Haron and friends [28] for optimization, the sequence of the probe with quenchers like FAMGAACGGCTGACC AAACT-BHQ-1 can be changed from the IS6110 gene TB probe. A 5 'end of the probe was labelled with 6-carboxy-fluorescence-dye (FAM), and non-fluorescence dye (BHQ-1) was labelled with a 3' end. Real-time PCR was subjected to the DNA from bacterial isolates and clinical samples using the TB primers and TB probe mixture. The reaction mixture consists of template DNA, TaqManMasterMix, primers and probe. Analysis was carried out at 60℃ for 1 min. The cycling parameters for Absolute Quantification were done at 50℃ for 2 min, 95℃ for 10 min, followed by 40 cycles of denaturation at 95℃ for 15 sec and annealing at 60℃ for 1 min. The data were collected during annealing steps.

Detection of Amplified DNA by Indigenous DNA detector System: Principal: Detector system is based on the Detection of the PCR products with the help of Precision machined micro well chip which operates on the principle of Laser or U.V induced Fluorescence detection system with the help of avalanche photodiodes (APDs) (Fig. 1). The system consists of a 450nm/488nm laser diode imaged onto the sample slide by a microscope objective. The fluorescence light from the sample after passing through a narrow band pass filter falls on the PMT. The output of the laser diode is polarized. The sample "slide" mounted on a linear stage is traversed across the focal spot simultaneously measuring the PMT output. The ratio of the fluorescence intensity from the sample trench to that from the reference trench is measure of the TB bacteria concentration. So after completion of PCR cycles of conventional machine, 2 µl of amplified sample was loaded on the biochip of the detection system and command was given to the system for scanning of the biochip in order to display the results, final results for samples were displayed on the mobile in form of peaks.

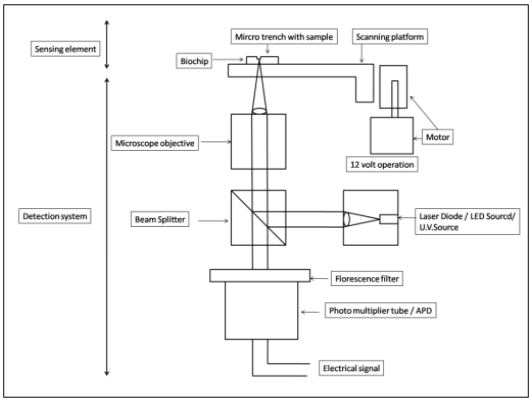

Fig. 1. Design of Indigenous DNA detector System Prototype.

If flat line was observed on mobile screen it suggest sample is negative and if sharp peak was observed on the system then sample is positive for the Tuberculosis

Statistical Analysis: MEDCALC® online statistical analysis software for analysis of Sensitivity and Specificity and accuracy of the indigenous detector system method were compared with that microscopy and RTPCR based Assay.

III. RESULTS AND DISCUSSION

AFB Microscopy: A total of 69 samples were analyzed out of which 24 samples were found positive by microscopy technique and 45 samples were found negative. The confirmation of positivity and negativity was confirmed by extensively analysing the stained smears under microscope, slide which shown red rod shaped bacilli with blue background, labelled as positive and the slides which don't showed red bacilli, labelled as negative for the tuberculosis. The sensitivity and specificity was calculated for microscopy by considering RT-PCR as a gold standard assay on MEDCALC® online statistical analysis software, the results were displayed in the Table 2.

Real Time PCR (Sybergreen based Assay): The real time PCR assay was performed for all collected 69 samples, out of which 38 samples shown successful amplification plot and 31 samples doesn't showed any amplification, which indicates that 38 samples were positive for Tuberculosis and 31 samples are negative. The conclusion of the samples were drawn on the basis of value of Threshold Cycle for all positive samples it ranges between 11 to 24 cycles and for negative samples no threshold value of cycles was obtained. The amplification plot for few batch of the samples are as shown in Fig. 2.

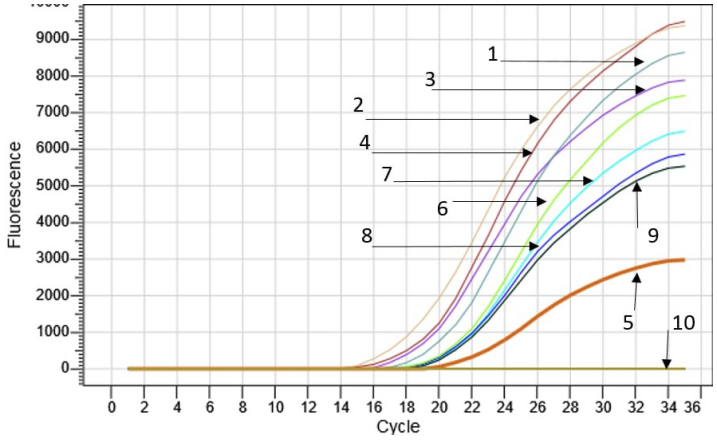

Fig. 2. Amplification plot for Real-Time PCR assay: Curve No. 1-9 denotes successful amplification of positive samples and No.10 denotes no amplification of negative samples.

The graph of Fluorescence verses cycle denotes the amplification plot for the amplified samples by Real Time PCR assay Fig. 1 showed amplification of nine samples out of 38 samples denoted by arrow 1- 9 and line denoted by arrow 10 represents no amplification plot for 12 samples as the results lies on same line. The sensitivity and specificity for Real Time assay was calculated by using MEDCALC® online statistical analysis software and shown in Table 2.

Indigenous TB detection system: After amplification of samples by using sybergreen based assay on conventional PCR machine, the samples are then loaded on the detection chip of the detector system and then left for final reading. The mobile connected to detection system displayed the results after analysing the samples as shown in Fig. 3. It denotes representative Images displayed on Mobile for Positive samples Denoted by Fig. 3-A and Negative Samples Fig. 3-B. Samples which shown sharp blue peak denoted by arrow p indicates the signal given by positive samples and on the other hand samples who do not displayed the peak instead showed flat line denoted by arrow *s* in Fig. 3-B indicates sample is negative for TB, arrow *q* indicates the red line which is sign that, system is scanning the loaded sample portion. A total of 39 samples found positive and 30 samples were found negative for the presence of TB by indigenous TB Detection system. The sensitivity and specificity for the test was calculated on MEDCALC® online statistical analysis software as shown in Table 2.

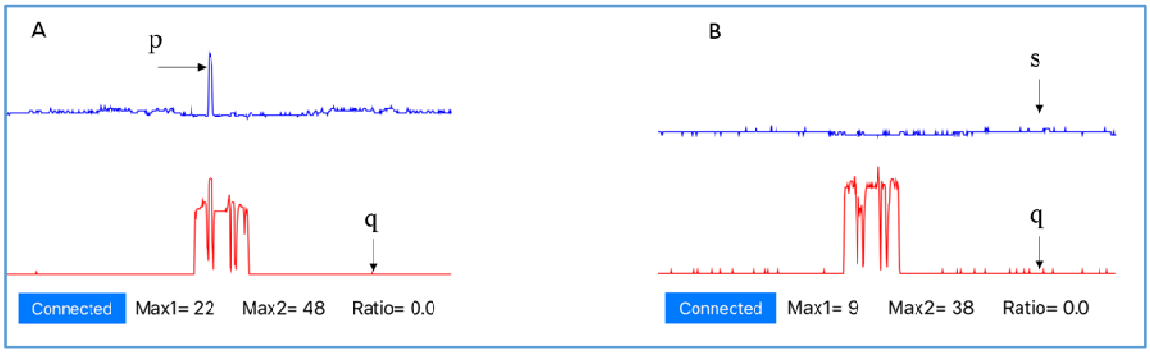

Fig. 3. Indeginouse DNA Detection System: 3A Results of Positive sample 3B Negative samples.

Statistical analysis for diagnostic test showed that Real Time PCR has high sensitivity (100%) and specificity (100%) for Detection of Tuberculosis, Smear microscopy has low sensitivity (63.16%) and high Sensitivity (100%) on the other hand Indigenous TB Detection has higher sensitivity (100%) and slightly low specificity (96.77%) with diagnostic accuracies of (100%), (83.13%) and (98.30%) respectively for Real Time PCR assay, AFB Microscopy and TB Detection system. The results for comparative statistical analysis are displayed in Table 2. Rapid identification of Mycobacterium infections is critical in clinical management of various diseases. It would determine the proper time for administration of antibiotics, the most suitable antibiotic, contact precautions and prophylaxis [29-32]. Many promising advances have been made in the development of novel tools to diagnose TB but they are still restricted to tertiary healthcare centres due to high cost [33]. In this report, novel methods of end point detection of amplified PCR product were address the challenges of MTB diagnosis. Our research highlights the sensitivity and specificity of indigenously developed TB detection system. The performance of the detection system was compared with other conventional methods used in the diagnosis of TB such as Smear microscopy technique and Real Time PCR based assay as described by previous studies [34]. Previous studies had shown that microscopy technique success is highly varies from 22% to 96% and several researchers rate it at round 60%, in present study we found that the success rate of the smear microscopy technique is 63.16 in terms of sensitivity towards diagnosis of TB [24, 35]. While microscopic smear testing is low-cost, fast and easy to conduct, it suffers from poor sensitivity and lack of distinctive specificity [36]. Conventionally amplified Nucleotide sequences by PCR are detected by expensive and labour intensive techniques which are highly expensive and also time consuming such as Gel electrophoresis. Real time PCR based assays can conveniently be used for diagnosis of TB in time bound manner as they have higher sensitivity and specificity as reported by previous studies, present study also reported that sensitivity and specificity of the Real Time PCR based assay is around 100%. Also present techniques for PCR Products detection uses expensive CCD cameras and Detectors which overall increases the cost of the equipment's and cost of the test per sample. Present invention addresses the Endpoint detection of PCR Products with the help of conventional low cost available fluorescent dyes on LED or U.V induced fluorescence detector system with avalanche photodiodes on Precision machined disposable micro well chip band 100% sensitivity and specificity in concurrence with RTPCR based assay. So the results of indigenously developed system found prominent for diagnosis of Tuberculosis with similar sensitivity and specificity as that of Real Time PCR assay and only differ in single sample results which may be due to unwanted signal given due to contamination in the sample.

Table 2: Results of statistical analysis for Real Time PCR, AFB Microscopy and TB detection system.

Statistics	Real Time PCR		AFB Microscopy (ZN- Staining)		TB Detection System	
	Value	95% CI	Value	95% CI	Value	95% CI
Sensitivity	100.00%	90.75% to 100.00%	63.16%	45.99% to 78.19%	100.00%	90.97% to 100.00%
Specificity	100.00%	88.78% to 100.00%	100.00%	92.13% to 100.00%	96.77%	83.30% to 99.92%
Positive Likelihood Ratio	*	*	*	*	31.00	4.51 to 213.18
Negative Likelihood Ratio	0.00	*	0.37	0.24 to 0.56	0.00	*
Disease prevalence (*)	55.07%	42.62% to 67.08%	45.78%	34.79% to 57.08%	55.71%	43.34% to 67.59%
Positive Predictive Value (*)	100.00%	*	100.00%	*	97.50%	85.01% to 99.63%
Negative Predictive Value (*)	100.00%	*	76.27%	67.95% to 82.98%	100.00%	*
Accuracy (*)	100.00%	94.79% to 100.00%	83.13%	73.32% to 90.46%	98.57%	92.30% to 99.96%

IV. CONCLUSION

Thus the device can be used for screening and diagnosis of infectious diseases specially TB in cost effective manner as it only requires the conventional amplification system instead of costly optical detection systems such as Real Time PCR and GenXpert systems. The only limitation associated with this study is we had only tested Pulmonary Tuberculosis cases and had not performed comparison with gold standard culture technique as it is time consuming as well as labor intensive, instead we had compared the results with highly sensitive and specific molecular biology based assay as Real Time PCR.

V. FUTURE SCOPE

To provide basic diagnostic facilities at resource limited settings, simple and cost effective solutions has to be developed which will be rapid and accurate, present system was developed to address these needs and a small initiative has been taken to evaluate this system for deployment process through mode of present study, thus in near future we are going to deploy the system for screening of suspects at rural setup after completion of all regulatory approvals needed for deployment of the system.

Conflict of Interest. The authors whose names are listed in author list declares that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the supported received from the MGM Institute of Health Sciences (MGMIHS) & Synergy Nanosystems for development and evaluation of this system.

REFERENCES

[1]. World Health Organization. (2015). *World health statistics 2015*. World Health Organization.

[2]. Bali, M., Sood, S., & Singh, P. S. (2009). Study of pyridine based triazole derivatives as Mycobacterium Tuberculosis TMPK Inhibitors. *International Journal of Theoretical & Applied Sciences*, *1*(2), 38-41.

[3]. Wani, I., & Chander, J. (2013). Incidence of multidrug-resistant (MDR) and extensively drug resistant (XDR) tuberculosis among different age groups in tertiary care hospitals of Chandigarh, India. In *Biological Forum*, *5*(1), 21-26.

[4]. Marais, B. J., Brittle, W., Painczyk, K., Hesseling, A. C., Beyers, N., Wasserman, E., & Warren, R. M. (2008). Use of light-emitting diode fluorescence microscopy to detect acid-fast bacilli in sputum. *Clinical infectious diseases*, *47*(2), 203-207.

[5]. Mugusi, F., Villamor, E., Urassa, W., Saathoff, E., Bosch, R. J., & Fawzi, W. W. (2006). HIV co-infection, CD4 cell counts and clinical correlates of bacillary density in pulmonary tuberculosis. *The International Journal of Tuberculosis and Lung Disease*, *10*(6), 663-669.

[6]. Sule, P., Tilvawala, R., Mustapha, T., Hassounah, H., Noormohamed, A., Kundu, S., & Cirillo, J. D. (2019). Rapid tuberculosis diagnosis using reporter enzyme fluorescence. *Journal of clinical microbiology*, *57*(12), 1-15.

[7]. CLSI. (2008). Laboratory Detection and Identification of Mycobacteria; Approved Guideline. CLSI document M48-A.

[8]. Kim, J. H., Kim, Y. J., Ki, C. S., Kim, J. Y., & Lee, N. Y. (2011). Evaluation of CobasTaqMan MTB PCR for detection of Mycobacterium tuberculosis. *Journal of clinical microbiology*, *49*(1), 173-176.

[9]. Prasad, K. D., & Murthy, M. B. R. (2019). A Method for Diagnosis of Tuberculosis using Combined Blur and Affine Moment Invariants on Cavity Image. *International Journal on Emerging Technologies*, *10*(2), 455–461.

[10]. Hendriksen, R. S., Le Hello, S., Bortolaia, V., Pulsrikarn, C., Nielsen, E. M., Pornruangmong, S., & Aarestrup, F. M. (2012). Characterization of isolates of Salmonella entericaserovar Stanley, a serovar endemic to Asia and associated with travel. *Journal of clinical microbiology*, *50*(3), 709-720.

[11]. World Health Organization. (2013). *Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB* (No. WHO/HTM/TB/2013.16). World Health Organization.

[12]. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., & Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, *239*, 487-491.

[13]. Li, H., Gyllensten, U. B., Cui, X., Saiki, R. K., Erlich, H. A., & Arnheim, N. (1988). Amplification and analysis of DNA sequences in single human sperm and diploid cells. *Nature*, *335*, 414-417.

[14]. Mullis, K. B., & Faloona, F. A. (1989). Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. In *Recombinant DNA Methodology,* 189-204.

[15]. Mullis, K. B. (1990). The unusual origin of the polymerase chain reaction. *Scientific American*, *262*(4), 56-65.

[16]. Law, J. W. F., AbMutalib, N. S., Chan, K. G., & Lee, L. H. (2015). Rapid methods for the detection of food borne bacterial pathogens: principles, applications, advantages and limitations. *Frontiers in microbiology*, *5*, 1-19.

[17]. Yamamoto, Y. (2002). PCR in diagnosis of infection: detection of bacteria in cerebrospinal fluids. *Clin. Diagn. Lab. Immunol.*, *9*(3), 508-514.

[18]. Erlich, H. A., Gelfand, D., & Sninsky, J. J. (1991). Recent advances in the polymerase chain reaction. *Science*, *252*, 1643-1651.

[19]. Murakami, K., Minamide, W., Wada, K., Nakamura, E., Teraoka, H., & Watanabe, S. (1991). Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. *Journal of clinical microbiology*, *29*(10), 2240-2244.

[20]. Chan, K., Wong, P. Y., Yu, P., Hardick, J., Wong, K. Y., Wilson, S. A., & Wong, S. S. (2016). A rapid and low-cost PCR thermal cycler for infectious disease diagnostics. *PLoS One*, *11*(2), 1-17.

[21]. Wong, G., Wong, I., Chan, K., Hsieh, Y., & Wong, S. (2015). A rapid and low-cost PCR thermal cycler for low resource settings. *PLoS one*, *10*(7).

[22]. Agrawal, N., Hassan, Y. A., & Ugaz, V. M. (2007). A pocket-sized convective PCR thermocycler. *AngewandteChemie International Edition*, *46*(23), 4316-4319.

[23]. Kent, P. T. (1985). *Public health mycobacteriology: a guide for the level III laboratory*. US Department of Health and Human Services, Public Health Service, Centers for Disease Control.

[24]. Gholoobi, A., Masoudi-Kazemabad, A., Meshkat, M., & Meshkat, Z. (2014). Comparison of culture and PCR methods for diagnosis of Mycobacterium tuberculosis in different clinical specimens. *Jundishapur journal of microbiology*, *7*(2). DOI: 10.5812/jjm.8939.

[25]. Eisenach, K. D., Donald Cave, M., Bates, J. H., & Crawford, J. T. (1990). Polymerase chain reaction amplification of a repetitive DNA sequence specific for Mycobacterium tuberculosis. *Journal of Infectious Diseases*, *161*(5), 977-981.

[26]. Peres, R. L., Maciel, E. L., Morais, C. G., Ribeiro, F. C. K., Vinhas, S. A., Pinheiro, C., & Palaci, M. (2009). Comparison of two concentrations of NALC-NaOH for decontamination of sputum for mycobacterial culture. *The International journal of tuberculosis and lung disease*, *13*(12), 1572-1575.

[27]. Kim, S. W., Kim, S. I., Lee, S. J., Lee, J. H., Ryu, Y. J., Shim, S. S., & Chang, J. H. (2015). The effectiveness of real-time PCR assay, compared with microbiologic results for the diagnosis of pulmonary tuberculosis. *Tuberculosis and respiratory diseases*, *78*(1), 1-7.

[28]. Haron, S., Issa, R., Sidik, N. M., & Zin, N. M. (2008). The usefulness of PCR amplification for direct detection of Mycobacterium tuberculosis DNA from clinical samples. *Biotechnology*, *7*(1), 100-105.

[29]. Mokaddas, E., & Ahmad, S. (2007). Development and evaluation of a multiplex PCR for rapid detection and differentiation of Mycobacterium tuberculosis complex members from non-tuberculous mycobacteria. *Japanese journal of infectious diseases*, *60*(2/3), 140.

[30]. Kox, L. F., Rhienthong, D., Miranda, A. M., Udomsantisuk, N., Ellis, K., Van Leeuwen, J., & Kolk, A. H. (1994). A more reliable PCR for detection of Mycobacterium tuberculosis in clinical samples. *Journal of Clinical Microbiology*, *32*(3), 672-678.

[31]. Kox, L. F., Rhienthong, D., Miranda, A. M., Udomsantisuk, N., Ellis, K., Van Leeuwen, J., & Kolk, A. H. (1994). A more reliable PCR for detection of Mycobacterium tuberculosis in clinical samples. *Journal of Clinical Microbiology*, *32*(3), 672-678.

[32]. Field, S. K., & Cowie, R. L. (2006). Lung disease due to the more common nontuberculous mycobacteria. *Chest*, *129*(6), 1653-1672.

[33]. Brock, I., Ruhwald, M., Lundgren, B., Westh, H., Mathiesen, L. R., & Ravn, P. (2006). Latent tuberculosis in HIV positive, diagnosed by the M. tuberculosis specific interferon-γ test. *Respiratory research*, *7*(1), 56.
[34]. Eisenach, K. D., Donald Cave, M., Bates, J. H., & Crawford, J. T. (1990). Polymerase chain reaction amplification of a repetitive DNA sequence specific for Mycobacterium tuberculosis. *Journal of Infectious Diseases*, *161*(5), 977-981.
[35]. Osman, A. L., Saeed, N. S., & Elhassan, M. M. (2014). Polymerase Chain Reaction targeting insertion sequence IS6110 for the diagnosis of pulmonary tuberculosis among Sudanese children and young adults. *International journal of mycobacteriology*, *3*(4), 252-258.
[36]. Ritis, K., Tzoanopoulos, D., Speletas, M., Papadopoulos, E., Arvanitidis, K., Kartali, S., & Sideras, P. (2000). Amplification of IS6110 sequence for detection of Mycobacterium tuberculosis complex in HIV-negative patients with fever of unknown origin (FUO) and evidence of extrapulmonary disease. *Journal of internal medicine*, *248*(5), 415-424.

How to cite this article: Patil, Y. and Thakur, M. (2020). Development and Validation of Indigenously developed DNA Detection System for *M. Tuberculosis*. *International Journal on Emerging Technologies*, *11*(3): 182–187.