The Role of TB-LAMP Method in Detecting *Mycobacterium tuberculosis* from Sputum of Patients Suspected of Having Pulmonary Tuberculosis

Mardiastuti H. Wahid[1], Agus Sjahrurachman[1], Ardath H. Sitorus[1], Erlina Burhan[2]

[1] Department of Clinical Microbiology, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo General Hospital, Jakarta, Indonesia.
[2] Department of Pulmonology and Respiratory Medicine, Faculty of Medicine Universitas Indonesia - Persahabatan Hospital, Jakarta, Indonesia.

Corresponding Author:
Mardiastuti H. Wahid, MD., PhD. Department of Clinical Microbiology, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo General Hospital. Jl. Pegangsaan Timur 16, Jakarta 10320, Indonesia. email: mardiastutiw@yahoo.com.

ABSTRAK

Latar belakang: *Indonesia merupakan salah satu negara dengan insiden tuberkulosis (TB) tertinggi di dunia. Penegakan diagnosis secara tepat termasuk ke dalam upaya untuk mengendalikan TB. World Health Organization telah merekomendasikan suatu Tes Cepat Molekuler, yaitu loop-mediated isothermal amplification (LAMP). Agar metode LAMP dapat diterapkan secara rutin, maka perlu dilakukan penelitian untuk mengevaluasi metode tersebut.* ***Metode:*** *penelitian ini merupakan studi potong lintang dan dilaksanakan di Laboratorium Mikrobiologi Klinik Fakultas Kedokteran Universitas Indonesia terhadap 100 pasien terduga TB paru. Setiap pasien menyerahkan dua sputum langsung. Terhadap setiap sputum langsung dilakukan pemeriksaan Basil Tahan Asam (BTA). Dua sediaan sputum langsung dari setiap pasien digabung untuk menghasilkan mixed sputum. Terhadap setiap mixed sputum dilakukan pemeriksaan BTA, biakan Lowenstein-Jensen dan TB-LAMP.* ***Hasil:*** *penelitian menunjukkan persentase hasil LAMP (+) pada mixed sputum dengan hasil BTA (-) sebesar 32,78%. Metode TB-LAMP memiliki nilai sensitivitas sebesar 100% (95% CI 89,56-100%) dan spesifisitas sebesar 69,64% (95% CI 55,74-80,84%). Nilai duga positif TB-LAMP sebesar 71,19% (95% CI 57,73-81,86%), sedangkan nilai duga negatif TB-LAMP sebesar 100% (95% CI 88,83-100%).* ***Kesimpulan:*** *metode TB-LAMP memiliki nilai sensitivitas dan nilai duga negatif yang tinggi.*

Kata kunci: *sputum, TB-LAMP, tuberkulosis.*

ABSTRACT

Background: *Indonesia is one of the countries with the highest incidence of tuberculosis (TB) in the world. Appropriate diagnosis is an effort to control TB. The World Health Organization has recommended loop-mediated isothermal amplification (LAMP). In order to be applied routinely, it is necessary to do research to evaluate the LAMP method.* ***Methods:*** *the research was a cross-sectional study and was carried out at the Clinical Microbiology Laboratory of the Faculty of Medicine, Universitas Indonesia, for 100 patients suspected of having pulmonary TB. Each patient handed over two direct sputum specimens. For each direct sputum specimen, an acid-fast bacilli (AFB) smear was carried out. Two direct sputum specimens from each patient were combined to produce mixed sputum. For each mixed sputum specimen, an AFB smear, Lowenstein-Jensen culture, and TB-LAMP were carried out.* ***Results:*** *the percentage of LAMP (+) cells in mixed sputum that was AFB (-) was*

32.78%. The TB-LAMP showed a sensitivity of 100% (95% CI 89.56–100%), a specificity of 69.64% (95% CI 55.74–80.84%), positive predictive value of 71.19% (95% CI 57.73–81.86%), and negative predictive value of 100% (95% CI 88.83–100%). ***Conclusion:*** *TB-LAMP has both high sensitivity and negative predictive value.*

Keywords: *sputum, TB-LAMP, tuberculosis.*

INTRODUCTION

According to the Global Tuberculosis Report published by the World Health Organization (WHO), in 2015, it is estimated that there were 10.4 million cases of pulmonary tuberculosis (TB) worldwide. Indonesia is second globally in terms of TB disease burden, with an average incidence of 395/100,000 individuals in 2015.[1] One indicator of TB control in Indonesia's health profile is the percentage of patients with bacteriologically confirmed pulmonary TB among all patients with recorded pulmonary TB (bacteriological and clinical). The indicator number is at least 70%. In 2015, the proportion of patients with bacteriologically confirmed pulmonary TB among all patients with pulmonary TB recorded/treated throughout Indonesia was 57.1%. This means that it has not reached the expected target.[2] Efforts to control TB, among others, include making an accurate diagnosis. To support the diagnosis of pulmonary TB, we need laboratory diagnostic tests, such as acid-fast bacilli (AFB) smear, culture, and molecular rapid test.[1,3]

The World Health Organization (WHO) has recommended the examination of two direct sputum specimens for patients with pulmonary TB.[4] In its implementation, subsequent visits to surrender sputum encountered several obstacles, including costs. The use of mixed sputum was deemed necessary to overcome the obstacles.[5]

Regarding available laboratory diagnostic tests, the need for trained operators is one of several limitations of the AFB smear. In addition, culture tests require a relatively long turnaround time. Molecular tests based on nucleic acid amplification techniques are generally complicated and require a lot of investment. A commercial molecular rapid test for TB based on loop-mediated isothermal amplification (LAMP), namely TB-LAMP (Eiken Chemical Company Ltd., Tokyo, Japan) has been developed to detect the *Mycobacterium tuberculosis* complex (MTBC).[6] LAMP is a nucleic acid amplification method using four different primers specifically designed to recognize six different regions of the target gene.[7] The target gene amplified by the LAMP method is gyrase subunit B (gyrB) and insertion sequence IS6110.[8] The amplification occurs at a constant temperature.[9]

In 2016, the WHO recommended the use of TB-LAMP to replace AFB smear in diagnosing adults with signs and symptoms of pulmonary TB. In addition, TB-LAMP can be considered as a further examination of the smear in adults with signs and symptoms of pulmonary TB, especially when further examination of sputum with a negative smear is needed.[1] We expect the TB-LAMP method provides benefits as a TB laboratory diagnostic modality, so we can use it in primary health care facilities in Indonesia. Therefore, we conducted a study to determine the effectiveness of TB-LAMP in detecting M. tuberculosis in sputum.

METHODS

We conducted this cross-sectional study at the Clinical Microbiology Laboratory, Faculty of Medicine, Universitas Indonesia, Jakarta, from July 2017 to February 2018. The study was declared to have passed ethical review (Ethical approval No. 547/UN2.F1/ETIK/2017; Date: June 12, 2017) by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia. Research participants were patients from health services in Jakarta, which were patients suspected of having pulmonary TB, patients that could spontaneously excrete sputum (volume 3–5 ml), and new patients with pulmonary TB. Patients suspected of having multidrug-resistant TB (MDR-TB) and patients who refused to take part in the study were excluded from the study. Calculation of sample size used a formula for a single proportion. The calculation also took into

account the proportion of patients with pulmonary TB with a positive AFB smear throughout Indonesia and the possibility of drop-out. A total of 100 patients were selected as research subjects. Each study subject handed over two direct sputum specimens (two spot sputum or spot-morning sputum specimens). For each direct sputum specimen, a smear was carried out. Two direct sputum specimens from each patient were then combined to produce mixed sputum. For each mixed sputum specimen, AFB smear, Lowenstein-Jensen (LJ) culture, and TB-LAMP were performed.

Microscopic and Culture

AFB smear examination was performed using Ziehl-Neelsen staining. Interpretation of the smear was carried out according to the International Union Against Tuberculosis and Lung Diseases (IUATLD) guidelines. The results of AFB smears in the direct sputum specimens was defined as positive when one or both of the sputum specimens showed a positive AFB smear. Meanwhile, if the two direct sputum specimens showed a negative smear, then the results of the AFB smear on direct sputum samples were declared negative.

Sputum was decontaminated using the Petroff method before being cultured. Culture was incubated for up to 8 weeks, and the growth of colonies was observed every week. If there was no growth within 8 weeks, the culture results were considered negative.[10] Cultures that were grown were then identified using the MPT64 test.

TB-LAMP Method

In this study, the LAMP test used a work kit (preparations-extractions-reactions) and TB-LAMP compact instrument (heating block-reaction block-fluorescent detector) developed by Eiken Chemical Company Ltd., Tokyo, Japan.[6] The TB-LAMP procedure was as described by the manufacturer.

Statistical Analysis

The obtained data was processed using SPSS version 22. Diagnostic tests were carried out to obtain the sensitivity and specificity of the LAMP method and to determine the positive predictive value and negative predictive value of the LAMP method.

RESULTS

Characteristics of Patients

The sputum that was included in this study was collected from patients who met the inclusion criteria. The characteristics of the

Table 1. Characteristics of Patients (n = 100).

Variable	n = %
Gender	
- Male	65
- Female	35
Age (years old)	
- < 25	5
- 25–44	34
- 45–64	49
- ≥ 65	12
Clinical complaints	
- Coughing (≥ 2 weeks)	100
- Hemoptysis	10
- Chest pain	7
- Shortness of breath	29
Chest X-rays	
- Fibroinfiltrate	9
- Infiltrate	37
- Infiltrate and cavity	2
- Cavity	3
- Opacities	1
- Consolidation	6
- N/A	42
Comorbidity	
- Asthma	2
- DM	20
- DM and hypertension	3
- Hepatitis B	1
- Hyperthyroidism	1
- Hypertension	3
- Hypertension and CAD	1
- N/A	32
- None	37
HIV status	
- N/A	71
- Negative	27
- Positive	2
AFB (+)	
- Spot Sputum	36
- Morning Sputum	40

N/A = Not Available; DM = Diabetes Mellitus; CAD = Coronary Artery Disease; HIV = Human Immunodeficiency Virus; AFB = Acid-Fast Bacilli.

Table 2. Comparison of AFB Smear on Direct Sputum and Mixed Sputum (n = 100).

	AFB on Direct Sputum			Kappa (κ) (95% CI)
	Positive	Negative	Total	
AFB on Mixed Sputum				
Positive	38	1	39	0.88 (0.78–0.97)
Negative	5	56	61	
Total	43	57	100	

AFB = Acid-Fast Bacilli

research subjects can be seen in **Table 1**. Each patient submitted two sputum specimens for this study. The first sputum was a spot sputum, while the second sputum could be morning sputum or spot sputum. In this study, all the second sputums delivered by patients were morning sputums.

Comparison of Microscopic Tests on Direct Sputum and Mixed Sputum

Comparison of the AFB smear result on direct sputum and mixed sputum can be seen in **Table 2**. To elucidate whether mixed sputum can represent two direct sputum specimens, the kappa test was carried out.

Comparison of AFB Smear and TB-LAMP

The results of AFB smears (direct sputum and mixed sputum) were then compared with the results of TB-LAMP. The results of AFB smears on direct sputum presented together with the results of TB-LAMP can be seen in **Table 3**.

In **Table 3**, it can be seen that there were 16 patients with negative AFB smear results on both direct sputums accompanied by positive results on the LAMP test. There were also 41 patients with negative AFB smear results accompanied by negative results on the LAMP test. The total number of patients in the two groups mentioned above was 57 people. Thus, the percentage of negative AFB smear results with positive LAMP results in all patients with negative AFB smear results was 28.07% (16/57).

Table 3. Comparison of AFB Smear on Direct Sputum and TB-LAMP (n = 100).

AFB (+) on two sputums with LAMP (+)	33 patients
AFB (+) on one sputum with LAMP (+)	10 patients
AFB (-) on two sputums with LAMP (+)	16 patients
AFB (-) with LAMP (-)	41 patients

AFB = Acid-Fast Bacilli; LAMP = Loop-Mediated Isothermal Amplification.

Besides using direct sputum, this study also used mixed sputum as an AFB smear material, then the results were compared with the results of the LAMP test obtained. **Table 4** shows the comparison in question. The percentage of negative smear results with positive LAMP results in all patients with negative AFB smear results was 32.78% (20/61).

Comparison of TB-LAMP and TB Culture Results

M. tuberculosis culture is the gold standard for microbiological examination of TB. Comparison of the results of TB-LAMP with LJ culture results can be seen in **Table 5**.

In all, 42 out of 100 (42%) samples showed positive culture results for *M. tuberculosis*. There were two samples with contaminated cultures

Table 4. Comparison of AFB Smear on Mixed Sputum and TB-LAMP (n = 100).

	LAMP		
	Positive	Negative	Total
AFB on mixed sputum			
Positive	39	0	39
Negative	20	41	61
Total	59	41	100

AFB = Acid-Fast Bacilli; LAMP = Loop-Mediated Isothermal Amplification.

Table 5. Comparison of TB-LAMP with *Mycobacterium tuberculosis* Culture.

	Mtb Culture			Sn % (95% CI)	Sp % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
	(+)	(-)	Total				
LAMP							
(+)	42	17	59	100	69.64	71.19	100
(-)	0	39	39	(89.56–100)	(55.74–80.84)	(57.73–81.86)	(88.83–100)
Total	42	56	98				

LAMP = Loop-Mediated Isothermal Amplification; Mtb = Mycobacterium tuberculosis; Sn = Sensitivity; Sp = Specificity; PPV = Positive Predictive Value; NPV= Negative Predictive Value.

in both LJ culture tubes. In addition, there were two samples with a contaminated culture in only one tube, while the results of culture on the other tubes were positive for *M. tuberculosis*. Thus, the total number of cultures that experienced contamination was six tubes. If the number is compared with the total number of culture tubes, which was 200 tubes, then the culture contamination rate was 3% (6/200).

Samples with contaminated culture in both culture tube were not included in the calculation for **Table 5**, whereas the sample with a contaminated culture in one culture tube only was still included in the table as intended.

Based on **Table 5**, it can be seen that there were 17 samples with positive results on LAMP, but with negative results on *M. tuberculosis* culture (referred to as samples with false positive results). Among the 17 samples, there were seven samples with positive results on LAMP, but culture growth was identified as non-tuberculous mycobacteria (NTM), while as many as 10 other samples were samples with positive results on LAMP, but there was no growth in culture.

DISCUSSION

As described in **Table 1**, it is seen that the number of men suspected of pulmonary TB (65%) is more than the number of women. In addition, it is also seen that the most frequent age group of patients is in the range of 25–64 years old. These results are in line with Indonesia's profile listed in the profile of 30 countries with high TB burdens issued by the WHO (2018) and data published by the Indonesian Ministry of Health's Data and Information Center (2018). These results showed that the incidence of TB was more prevalent in men and in the productive age group.[11]

Based on the most frequent clinical complaints, prolonged coughing, shortness of breath, and hemoptysis are the typical clinical complaints. These clinical symptoms were similar to the ones in the National Guidelines for Tuberculosis Control.[3]

Microscopic comparison of the direct sputum and mixed sputum (**Table 2**) had a kappa value of 0.88 ($p < 0.001$). Thus, the result of AFB smears performed on direct sputum was proven to be matched with the mixed sputum and statistically significant.

The percentage of positive LAMP results on direct sputum with negative AFB smear results was 28.07% (16/57). It seems that the results of our study showed a higher percentage compared to the research conducted by Kim et al.[12], which was 7.5%.Meanwhile, our study was in line with the study conducted by Mitarai et al.[13], which showed that 33 samples (25%) were positive LAMP out of 132 samples with negative AFB smears.

The clinical sensitivity of the AFB smear has a great variety of range, which is between 20% and 80%, with sensitivity being lower in paucibacillary cases, for example, in immunocompromised patients, whose bacterial numbers are less than 5,000–10,000 AFB/mm of sputum.[14] The sensitivity of the LAMP test is equal to 100–1,000 colony forming unit/ml.[15] Meanwhile, a study conducted by Bentaleb et al.[16] showed that the limit of detection (LOD) of LAMP was 10 copies of insertion sequence IS6110. Therefore, the arguments mentioned above might provide an explanation of the higher number of positive results obtained through the LAMP test compared to the results obtained through AFB smear (**Table 3**).

As described above, the use of mixed sputum is expected to increase positive results. The percentage of positive LAMP results was 32.78% (20/61) in mixed sputum with negative AFB smear results (**Table 4**), which is higher than the percentage of positive LAMP results (28.07%, 16/57) in direct sputums with negative AFB results (**Table 3**), but in fact, these data are not comparable, as both do not have basic equality. The LAMP test was only done using mixed sputum material, rather than direct sputum material (spot sputum or morning sputum).

TB-LAMP should be compared with TB culture as a gold standard. Sensitivity, specificity, PPV, and NPV of TB-LAMP was 100%, 69.64%, 71.19%, and 100%, respectively. In a study conducted by Alom et al.[17], it was clearly illustrated that the sensitivity of TB-LAMP was 100%, while the specificity was 42.85%. It seems that the results of this study are relatively in line with the results of the study by Alom et al.[17], even the specificity in this study was higher than the specificity obtained by Alom et al.[17] The research conducted by Senarath et al.[18] obtained a lower sensitivity (92%) and specificity (7%).

Unfavourable result of specificity in this study is related to false positive results. There are several possible causes of false positive events. First, there is contamination, which can be avoided by replacing new reagents, instruments, and equipment for LAMP work, along with more accurate laboratory practices.[7] LAMP reactions are very sensitive and contamination of small amounts of amplification products can cause false positive results. Several things have been done regarding this situation, such as cleaning the bench using 0.5% sodium hypochlorite before working on the test, separating the LAMP area from sputum handling area, changing gloves after removing the sputum or when in contact with the DNA solution, preparing a reaction tube for positive control in the last sequence after preparing a reaction tube for the sample and negative control, and spinning down the tube containing the positive control before opening it then immediately closing it again after the positive control has been released.[8] Implementing the above steps accurately, especially in peripheral health care facilities that are expected to perform LAMP tests, will provide better results. In addition, during the specimen preparation step, use of the Eiken pipette, which has a small tip (to transfer 60 µl of sputum from a sputum pot into a heating tube), might contribute to contamination. In the writings of Sartorius,[19] the selection of pipette tips according to the application can avoid the formation of aerosols when pipetting, so contamination can be avoided. Therefore, in the future, it is hoped that the availability of Eiken pipettes with larger tip holes can minimize the potential for aerosol formation when pipetting, to avoid possible contamination. Second, according to the study results of Wang et al.[20], the high primer concentrations that are used increase the likelihood of non-specific amplification induced by the formation of primer dimers. Third, according to the study results of Gray et al.[9], there is the possibility of a relationship between false positives and low reaction volume. The explanation given by Gray et al.[9] as to this is that the lower the volume of the reaction is, the higher the concentration of the reagent, causing self-priming, especially at increasing temperatures. Fourth, highly efficient amplification in the LAMP method, which is 10^9–10^{10} times in 15–30 minutes,[6] can also increase the likelihood of non-specific amplification. Fifth, according to the study results of Suleman et al.[21] the possibility of partial hybridization of one or more LAMP primers to DNA fragments, which are then randomly amplified by DNA polymerase enzymes. Lastly, according to Liu et al.[22], the indirect detection technique in the LAMP method, which uses the calcein/manganese ion, can not distinguish between desired amplification products and non-specific amplification products, which can lead to false positives.

The TB-LAMP detection kit used in this study uses the gyrB and insertion sequence IS6110 as the amplification target gene.[8] The sequences of the four different primers used are patents from LAMP companies (Eiken Chemical Company Ltd., Tokyo, Japan). Bi et al.[23] conducted a LAMP-based test with the hspX target gene to detect most of the MTBCs, since many pathogenic mycobacteria other than

those included in the MTBC (e.g., *M. kansasii*, and *M. marinum*) have no similar sequences with MTBC species. Therefore, Bi et al.[23] succeeded in detecting the majority of MTBC members without showing a positive reaction in the majority of pathogenic non-tuberculous mycobacteria (NTM) and non-mycobacteria species (e.g., M*ycoplasma pneumoniae* and *Haemophilus influenzae*), which can cause pulmonary infections.

Monitoring the rate of culture contamination in the mycobacteriology laboratory is a fundamental quality indicator. The rate of contamination reflects the laboratory's ability to process specimens. In this study, the rate of culture contamination (LJ solid media) that occurred was low (3%). Although, we have to consider it to guarantee the quality of the examination.

The WHO groups recommended the use of a number of Mtb diagnostic tests for various levels of the health care system. At primary level health care facilities, there are two recommended test formats: microscopic and LAMP examinations. Both formats should be used for the purpose of diagnosing active TB. The treatment monitoring of active TB can only be performed using the microscopic examination format.[24]

As a laboratory diagnostic test, TB-LAMP has several advantages, such as requiring minimal instrumentation,[25] short turn-around time (TAT) (< 2 hours), less practical work, visible results, and relatively low costs.[26] Cost per LAMP test is US$ 9.98. The price is cheaper than the cost per Xpert MTB/RIF test, which is US$ 13.38.[27] In addition, the LAMP test can also provide a greater number of test results, because it can work up to 14 samples for each test run.[25] Disadvantages of the TB-LAMP method include the potential for cross-contamination and errors in interpreting fluorescent results.[27]

CONCLUSION

The TB-LAMP method has a high sensitivity and NPV, despite unfavourable specificity and PPV. The results also showed that the positive results obtained through TB-LAMP were higher than the AFB smears in sputum. The TB-LAMP has a high sensitivity on direct sputum with AFB smear results that are concordant with TB culture, as well as AFB smear results that are discordant with TB culture.

ACKNOWLEDGMENTS

The authors wish to thank Budi Haryanto, MD, Clinical Microbiologist, for his assistance with the collection of specimens. Diagnostic kits and reagents were supplied by human.de, while consumables were supported by PT Sinergi Indonesia.

REFERENCES

1. World Health Organization. Global Tuberculosis Report. Geneva: World Health Organization; 2016. p. 1-201.
2. Ministry of Health The Republic of Indonesia (Kementerian Kesehatan Republik Indonesia). Indonesian Health Profiles (Profil Kesehatan Indonesia). Jakarta; 2015. p. 1-222. Indonesian.
3. Directorate General for Disease Control and Enviromental Health Ministry of Health The Republic of Indonesia (Direktorat Jenderal Pengendalian Penyakit dan Penyehatan Lingkungan Kementerian Kesehatan Republik Indonesia). National Guideline for Tuberculosis Control (Pedoman Nasional Pengendalian Tuberkulosis). Jakarta; 2014. p. 1-150. Indonesian.
4. World Health Organization. Implementing tuberculosis diagnostics: policy framework. Geneva: World Health Organization; 2015. p. 1-39.
5. Datta S, Shah L, Gilman RH, Evans CA. Comparison of sputum collection methods for tuberculosis diagnosis: a systematic review and pairwise and network meta-analysis. Lancet Glob Health. 2017;5(8):e760–e771.
6. World Health Organization. The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis: Policy guidance. Geneva: World Health Organization; 2016. p. 1-38.
7. Eiken Chemical Co. Ltd. Eiken Genome Site: Design of primers [Web page]. 2005. Available from: http://loopamp.eiken.co.jp/e/lamp/primer.html. Cited July 4, 2019.
8. Eiken Chemical Co. Ltd. Loopamp MTBC Detection Kit 380236–A Ref 972000 [Insert]. Japan; 2015.
9. Gray CM, Katamba A, Narang P, et al. Feasibility and operational performance of tuberculosis detection by loop-mediated isothermal amplification platform in decentralized settings: results from a multicenter study. J Clin Microbiol. 2016;54(8):1984–91.
10. Directorate General for Medical Service Ministry of Health The Republic of Indonesia (Direktorat Jenderal Bina Upaya Kesehatan Kementerian Kesehatan Republik Indonesia). Technical procedures for

Mycobacterium tuberculosis culture, identification and drug susceptibility test in solid media (Petunjuk teknis pemeriksaan biakan, identifikasi, dan uji kepekaan Mycobacterium tuberculosis pada media padat). Jakarta; 2012. p. 1-114. Indonesian.

11. World Health Organization. Global Tuberculosis Report. Geneva: World Health Organization; 2018. p. 1-231.
12. Kim CK, Cho EA, Shin DM, Choi SW, Shin SY. Comparative evaluation of the loop-mediated isothermal amplification assay for detecting pulmonary tuberculosis. Ann Lab Med. 2018;38(2):119–24.
13. Mitarai S, Okumura M, Toyota E, et al. Evaluation of a simple loop-mediated isothermal amplification test kit for the diagnosis of tuberculosis. Int J Tuberc Lung Dis. 2011;15(9):1211–7.
14. Gordillo-Marroquín C, Gómez-Velasco A, Sánchez-Pérez H, et al. Magnetic nanoparticle-based biosensing assay quantitatively enhances acid-fast bacilli count in paucibacillary pulmonary tuberculosis. Biosensors (Basel). 2018;8(4):128.
15. Jankovic VK, Furci L, Cirillo DM. Microbiology of Mycobacterium tuberculosis and a new diagnostic test for TB. In: Lange C, Migliori GB, editors. European respiratory monograph. Sheffield: European Respiratory Society; 2012. p. 1-13.
16. Bentaleb EM, Abid M, Messaoudi MDE, et al. Development and evaluation of an in-house single step loop-mediated isothermal amplification (SS-LAMP) assay for the detection of Mycobacterium tuberculosis complex in sputum samples from Moroccan patients. BMC Infect Dis. 2016;16(1):517.
17. Alom S, Mondal KJ, Tarafder S, et al. Evaluation of loop mediated isothermal amplification (Lamp) assay for diagnosis of Mycobacterium tuberculosis in sputum samples. IOSR-JDMS. 2018;17(6):63–8.
18. Senarath K, Usgodaarachchi R, Navaratne V, et al. Non specific amplification with the LAMP technique in the diagnosis of tuberculosis in Sri Lankan settings. J Tuberc Res. 2014;2(4):168–72.
19. Sartorius. Preventing Contamination in Pipetting [Web page]. 2016. Available from: https://www.labmanager.com/laboratory-technology/2016/02/preventing-contamination-in-pipetting#.XR7gH-gzbIU. Cited July 5, 2019.
20. Wang D, Brewster J, Paul M, Tomasula PM. Two methods for increased specificity and sensitivity in loop-mediated isothermal amplification. Molecules. 2015;20(4):6048–59.
21. Suleman E, Mtshali M, Lane E. Investigation of false positives associated with loop-mediated isothermal amplification assays for detection of *Toxoplasma gondii* in archived tissue samples of captive felids. J Vet Diagn Invest. 2016;28(5):536–42.
22. Wei L, Huang S, Liu N, et al. Establishment of an accurate and fast detection method using molecular beacons in loop-mediated isothermal amplification assay. Sci Rep. 2017;7:40125.
23. Bi A, Nakajima C, Fukushima Y, et al. A rapid loop-mediated isothermal amplification assay targeting hspX for the detection of Mycobacterium tuberculosis complex. Jpn J Infect Dis. 2012;65(3):247–51.
24. World Health Organization. World Health Organization model list of essential in vitro diagnostics. 1st ed. Geneva: World Health Organization; 2018. p. 1-28.
25. Pham TH, Peter J, Mello FCQ, et al. Performance of the TB-LAMP diagnostic assay in reference laboratories: results from a multicentre study. Int J Infect Dis. 2018;68:44–9.
26. Nakiyingi L, Nakanwagi P, Briggs J, et al. Performance of loop-mediated isothermal amplification assay in the diagnosis of pulmonary tuberculosis in a high prevalence TB/HIV rural setting in Uganda. BMC Infect Dis. 2018;18(1):87.
27. Nliwasa M, MacPherson P, Chisala P, et al. The sensitivity and specificity of loop-mediated isothermal amplification (LAMP) sssay for tuberculosis diagnosis in adults with chronic cough in Malawi. PLoS One. 2016;11(5):e0155101.