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Portable and Battery-Operated Isothermal Amplification Device Validation for Onsite Analysis of *M. tuberculosis* “DNA Hunter”

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ABSTRACT

Point-of-care (POC) devices play an important role in the protection of public health by providing rapid diagnosis of infectious diseases, patient management, and effective treatment. Fast, easy-to-interpret, environmentally resistant, and cost-effective POC tests that can be used practically in the field are gaining more and more importance every day. There is a need for portable devices that will enable rapid diagnosis kits to be used in the field for early diagnosis and treatment. The aim of this study is to evaluate the DNA hunter device that was developed in terms of providing the required temperature for *M. tuberculosis* (MTB) diagnosis of the loop-mediated isothermal amplification (LAMP) assay and visually evaluating the analysis results. The device in this study; handheld (total weight 430 g, outer dimensions 70 x 175 x 80 mm), the average operating time can reach a maximum temperature of 110 degrees in 2 minutes with a fully charged battery, and the processing time is about 90 minutes without being connected to electricity. It can display the pre-evaluation result on the screen with the full digital color sensor. The device can be adjusted to the desired reaction temperature and time. It also has software where sample registration numbers can be entered. DNA Hunter can be used for all analyses performed by the LAMP method and the results can be evaluated colorimetrically, thus it is well suited for POC testing.

Keywords: handheld device; loop-mediated isothermal amplification (LAMP); *M. tuberculosis* (MTB); Point of care (POC)

Highlights: A portable device has been developed that allows an important public health pathogen such as tuberculosis infection to be screened with the LAMP method without the need for complex laboratory infrastructure. The most important aspect of this device is that it is small enough to fit in the palm and can work independently of electricity for a certain period of time.

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INTRODUCTION

Analyses that can be performed quickly outside of the laboratory are known as Point-of-Care (POC) tests.¹ POC tests are required for disease screening in the diagnosis of infectious diseases, particularly in countries with limited laboratory facilities, a high disease burden, and a low income. The need for POC tests for the rapid screening of infectious diseases is rapidly expanding.² The main advantages of POC tests are to reduce procedures, and costs associated with hospitalization and prevent the risk of hospital-acquired infections by determining the infection factor detected during hospitalization, rapid diagnosis in epidemics and pandemics, and the ability to work with fewer samples compared to traditional methods.³

POC tests, as well as antimicrobial use control, rapid treatment initiation, and outbreak monitoring and control, all contribute to the investigation of unknown pathogens.⁴ With a compound annual growth rate of 11.4%, the global POC market was valued at USD 29.5 billion in 2020 and is expected to reach USD 50.6 billion in 2025. The production of devices for POC analysis has economic and commercial importance, as shown by these statistics.⁵ Tuberculosis is one of the oldest known diseases, is an infectious disease caused by the *Mycobacterium tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*). This disease is characterized by the presence of granulomas in infected tissues involving the respiratory tract or other organs. Although tuberculosis has fluctuated in its incidence over thousands of years of human history, it has remained a permanent threat to public health.⁶ Tuberculosis disease treatment takes a long time, it can be transmitted from patients with positive sputum smears via respiration to healthy people and can cause mortality. The fight against tuberculosis requires a continuous and disciplined public health practice. Because of droplet infection, each patient

should be diagnosed early and treated effectively to protect public health.^{7,8} Nucleic acid amplification methods are widely used to identify *M. tuberculosis* (MTB), which is difficult to see by microscopy and takes a long time to produce in culture.^{9,10} In parallel with the developments in molecular techniques such as Polymerase chain reaction (PCR), real-time PCR, and transcription-based amplification (TMA) have been developed for the diagnosis of tuberculosis.^{11,13} However, the most important disadvantage of molecular methods is that they mostly require a laboratory environment and cannot be applied as the POC tests.^{14,16}

Nucleic acid amplification tests (PCR, Real-Time PCR) can detect trace amounts of genetic material (DNA or RNA) of various pathogens in the early stage of the disease. However, the thermal cycling condition adds complexity to the way PCR devices operate.^{15,17} Recently, various isothermal amplification methods have been developed, such as rolling circle amplification (RCA), recombinase polymerase amplification (RPA), and loop-mediated isothermal amplification (LAMP). Among these methods, LAMP is the most popular isothermal nucleic acid test for detecting viruses, bacteria, fungi, and parasites due to its low cost and operation at a single temperature.^{16,19}

Loop-mediated isothermal amplification (LAMP) reaction is performed with four or six primer sets for DNA/RNA amplification.¹⁸ The most important advantage of the LAMP method is; It provides the opportunity to reproduce target nucleic acid sequences under isothermal conditions (60-65°C) in a miniaturized environment with low energy consumption.²⁰ Proliferation; turbidity can be monitored with dyes that show the amount of fluorescence or free magnesium bound to nucleic acids. Therefore, it does not require any expensive device, allowing the results to be evaluated with a simple optical system or with the naked eye. The LAMP method gives more sensitive results than other amplification

methods because it provides sequence-specific visual detection of the 4/6 region on the target gene.²¹ However, LAMP assay requires a heating block system in order to be operated as in other diagnostic techniques. Therefore, new generation smart devices are needed to perform analysis in places without laboratory infrastructure. The portability of these devices, low cost, robustness, ease of use, less need for trained personnel, easy-to-interpret results, and ability to produce accurate and reliable results quickly are important.²²

The aim of this study was to develop a hand-held portable device (DNA Hunter) for rapid and accurate diagnosis of MTB and enabling analysis without the expert in the field as point-of-care testing. DNA Hunter (total weight 430 g, outer dimensions 70 x 175 x 80 mm) can be adjusted to the desired reaction temperature and time also has software where sample numbers can be entered. The device has 6 aluminum chambers and the average operating time can be realized around 90 minutes with a fully charged battery, without being connected to electricity. Reservoir and the cover section have a heating function reaching a maximum temperature of 110°C in 2 minutes. The color change is measured and evaluated positively and negatively by a full digital color sensor and the result can be displayed and compared with the reference values on the screen.

MATERIALS AND METHODS

Materials

The 93 sputum samples (68-culture positive, 25-culture negative) were used in this study, and the *M. tuberculosis* H37Rv Pasteur Institute standard strain was provided by Atatürk Chest Diseases Hospital within the scope of the TUBITAK project (115R002). ARB staining, Löwenstein-Jensen culture (BD, New Jersey, USA), and Geneexpert (Cepheid, California, USA) analyses were routinely performed by the institutional laboratory where sputum samples were obtained.

The QIAamp DNA mini kit was purchased from (QIAGEN, Hilden, Germany). LAMP amplification reagents (WarmStart Colorimetric LAMP 2X Master Mix) were purchased from New England Biolabs (Massachusetts, USA) and Loopamp MTBC Detection Kit was purchased from Eiken Chemical Co., Ltd. (Tokyo, Japan). LAMP primers used in this study were synthesized Microsynth AG (Balgach, Switzerland) as HPLC grade.

Methods

LAMP primers targeted specifically for the MTB IS6110 gene (GenBank accession number: X17348) were designed using the PrimerExplorer V5 program. The primers were optimized according to the protocol described in the previous study.²³ The LAMP primers consisted of two outer primers (F3 and B3) and two inner primers [FIP (F1c + F2) and BIP (B1c + B2)], and two loop primers [FLP: (forward loop primer) and BLP (backward loop primer)].²¹

For the preparation of samples, the standard strain of *M. tuberculosis* H37Rv was used. H37Rv pure culture produced in Lowenstein Jensen (LJ) broth was homogenized with PBS (pH 6.8) in a sterile glass beaded tube, its density was adjusted according to McFarland 1 and accepted as the main dilution. It was then diluted up to 10¹ in 10-fold serial dilutions starting with the main dilution. ARB negative sputum samples were spiked with main stock and its serial dilution from 1/10 to 1/100000. A nonspiked sputum sample was used for negative control. DNA of all sputum samples was extracted using QIAamp DNA mini kit, according to the manufacturer's instructions. The purity and quality of DNA were controlled using the Implen NanoPhotometer (Implen GmbH, Germany). Then extracted DNA was stored at -20°C until used. For the colorimetric assay, NEB Warmstart colorimetric LAMP 2X master mix kit (Table 1) was used and the process was performed according to the manufacturer's instructions.

Table 1. Colorimetric LAMP Reaction Mixture

Content	Stock Const.	Final Const.	1X (µl)
2x Master mix	2 µL	1	12,5
10x Primer Mix	10 µL	1	2,5
DNA	40 ng/µL		1
Ultrapure water			9
Total			25

The limit of detection (LOD) of the *M. tuberculosis* LAMP test was determined as 10^2 CFU/mL in the previous study.²³ In this study to test whether the DNA hunter device performed the LAMP reaction correctly, five different concentrations (10^1 - 10^5 CFU/mL) were studied above and below the LOD limit. A bacteria-free sputum sample was used for negative control. The colorimetric LAMP method works on the principle of changing the color of the pH indicator dye added to the reaction with the hydrogen ions formed during the reaction changing the pH of the environment.²⁴ LAMP results were evaluated based on color change, with yellow color indicating positive and pink color examined as negative in visual evaluation. However, the pH-sensitive dye in the colorimetric LAMP reaction mix may not be seen as clear yellow or pink due to the amount of nucleic acid in the sample or some substances that come with the sample. The especially orange color formation can cause problems in evaluating the test. After LAMP reaction orange color is formed, which is considered an intermediate result in some studies even though the reaction color change from pink to yellow was not clear. In order to interpret

the unclear results, a color scale was created and shown in Figure 1.^{25,26}



Figure 1. LAMP Reaction Results; Positive: Yellow, Negative: Pink

(--) Strongly Negative, (-) Negative, (±) Positive/ Negative (Unclear), (+) Positive, (++) Strongly Positive.^{25,26}

Fabrication of DNA Hunter Device

The LAMP method requires a constant temperature (approximately 65 degrees) during the analysis. In the first design of the device, Peltier was used as the heater. However, the Peltier tends to generate an excessive amount of heat, so instead of using the Peltier as a heater, it was decided to use a resistor. The heater is designed to reach a maximum temperature of 110°C in 2 minutes at room temperature. The mechanical and electronic materials used were chosen to withstand temperatures of 150°C for a short period of time (maximum 2 minutes) and 120°C for an indefinite time. The temperature resistance between the top of the heater and the cover is very high. In order to keep the top cover temperature low a Teflon plate with a thickness of 4 mm was used. It is one of the materials with a very low thermal conductivity coefficient ($5 \cdot 10^{-4}$ Cal/c m. s. degree).

To prevent the heat from affecting the motherboard and graphics cards, the internal heat dissipation should be designed well. For this reason, the device's internal structure was changed several times and the air outlet was adjusted with the appropriate fan placement. Active cooling time was measured as 30 seconds while the fans were running, and the cooling time on its own (passive) was more than 10 minutes due to thermal insulation. It was observed that the warm-up cooling time was less than 2 minutes between 20 degrees and 60 degrees. DNA Hunter device was designed with 6 wells using Solidworks® software (DSS Solidworks 2016) and shown in Figure 2.

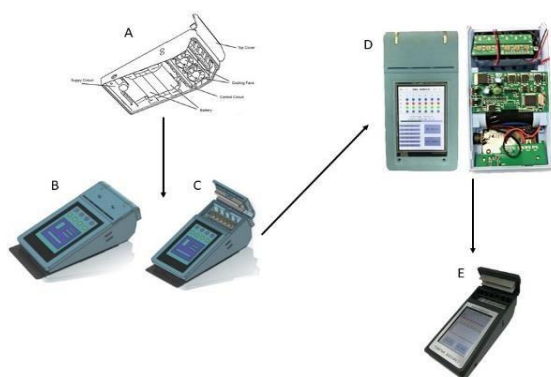


Figure 2. Design of the device (A), off mode (B), and open mode (C), processor card of the device system (D), and the completed device (E)

RESULTS AND DISCUSSION

Battery and Display Features

Since DNA Hunter was designed to be handheld, it was equipped with a Li-Ion battery with a low power consumption feature. With a fully charged battery, the total power drawn was about 10 W on average, and the running time was about 90 minutes. Every measuring cell was equipped with six high-precision full-color digital sensors. As a result of the system's evaluation, the color

change was measured during the test and compared to the reference values that were displayed on the screen.

The total weight of the unit is 430 g, and the exterior dimensions are 70 x 175 x 80 mm. The device does not require expertise to use; test protocols can be accessed by simply entering the transaction code, thanks to the 15 different protocol storage processes. It assures also three different temperature set values and warm-up times for each test process. The user is also provided with temporary or permanent correction options.

Test Program Features

Protocols can be quickly accessed by entering transaction codes, owing to the program's memory capacity for 15 different operations. The protocols determine three separate temperature set values and warm-up times for each test process (Figure 3).

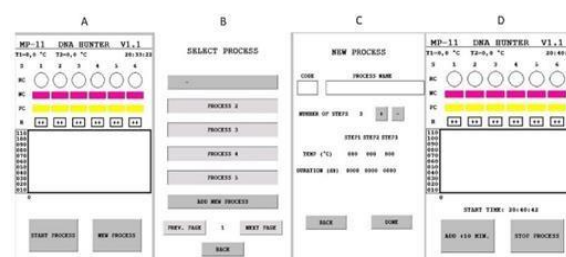


Figure 3. Program features (A) and (B), process selection page (C), and adding new process (D)

The user is given the option of temporary or permanent correction if appropriate. The results of the tests can be transferred to a microSD card and then to the host machine. In order to enter sample information on the device, a touch screen suitable for alphanumeric information entry and appropriate software was arranged. Test recipes and procedures can be entered on the device with the help of the same touch screen. In order to record the date and time of the tests, a battery-protected real-time clock was used.

Monitoring Temperature Changes of DNA Hunter

MTB-LAMP assay requires a constant temperature which is at 65°C for 30 minutes (Figure 4). To check the temperature stability of the device, every 6 wells were tested separately and also 10 repetitions were read for each well. Temperature adjustment is provided in the device with an accuracy of $\pm 0.1^\circ\text{C}$ between 50-100°C.

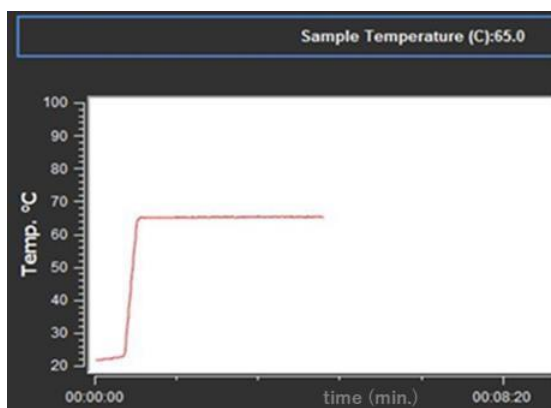


Figure 4. Temperature Curve of “DNA Hunter” Device

After 30 minutes, the device turned off the heating and indicated the end of the operation with an alarm sound before turning itself off at the end of the run.

Visualization of MTB

The colorimetric LAMP method works on the principle of changing the color of the pH indicator dye depending on the hydrogen ions formed during the reaction. In this study, a colorimetric LAMP master mix kit was used according to the optimized protocol.²³ LAMP assay was performed by a thermal cycler (Bio- Rad, Hamburg, Germany) at the same time as carrying out the DNA Hunter device. LAMP reaction operated under the same conditions (65°C for 30 minutes). As shown in Figure 1 the color change from pink to yellow in the tubes was considered positive, and the absence of color change (pink) was considered negative at the end of the result.²⁴ In our study, (orange), that is, unclear color formation, which creates problems in the evaluation of the results colorimetrically, was not observed.

LOD values on both devices were remarkably similar, which was 10^2 CFU/ml to monitor the performance stability of DNA Hunter 6 PCR wells in the device, were studied with samples contaminated with 5 different concentrations of bacteria, to evaluate whether there was a performance difference between the wells.²³ For this study, 10 readings were repeated for each well. The performance measurement chart is shown in Table 2.

Table 2. Performance Evaluation Results of the Device Wells

	1st Well					2nd Well					3rd Well					4th Well					5th Well					6th Well									
★	▼	■	●	□	△	▼	■	●	□	△	▼	■	●	□	△	▼	■	●	□	△	▼	■	●	□	△	▼	■	●	□	△					
1	-	-	+	+	+	-	-	-	+	+	-	-	+	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
2	-	-	-	+	+	-	-	-	+	+	-	-	+	+	+	-	-	-	+	+	-	-	+	+	+	-	-	+	+	+	-	-	+	+	+
3	-	-	-	+	+	-	-	-	+	+	-	-	+	+	+	-	-	-	+	+	-	-	+	+	+	-	-	+	+	+	-	-	+	+	+
4	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
5	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
6	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
7	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
8	-	-	+	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
9	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
10	-	-	-	+	+	-	-	+	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+

★ Number of readings, ▼ (- -) Strongly Negative,

■ (-) Negative, ● (±) Unclear, □ (+) Positive,

△(++) Strongly Positive

Sample Analysis

ARB staining, Löwenstein-Jensen culture, and Genexpert analyses were performed by the institutional laboratory where sputum samples were obtained. These sputum samples were subjected to DNA extraction using the QIAamp DNA mini kit in our laboratory. All isolates were analyzed in parallel with the thermal cycler using the Eiken Loopamp kit and the DNA hunter using the in-house LAMP method.²³ The results were demonstrated in Table 3 and Table 4.

Table 3. Sputum Sample Results

Sample No	*ARB	**LJ Culture	Gene Expert	Eiken Loopamp/ Thermal cycler	Inhouse LAMP/ DNA Hunter
1	++	+	+	+	+
2	+	+	+	+	+
3	++	+	+	+	+
4	-	-	-	-	-
5	++	++	+	+	+
6	+	++	+	+	+
7	++	++	+	+	+
8	++	++	+	+	+
9	-	-	-	-	-
10	-	-	-	-	-
11	++	+	+	-	+
12	++	++	+	-	-
13	++	+	-	-	+
14	+	++	+	-	+
15	++	+	+	+	+
16	++	++	+	+	+
17	-	-	-	+	-
18	++	++	+	+	+
19	++	+	-	-	-
20	++	+	+	+	+
21	++	+	+	+	-
22	+	-	+	+	+
23	-	-	-	-	-
24	++	+	+	+	+
25	+	+	-	+	+
26	+	++	+	+	+
27	+	-	+	+	+
28	-	-	+	+	+
29	++	+	-	+	+
30	++	+	-	+	+
31	-	-	-	-	-
32	-	-	-	-	-
33	++	+	+	+	+
34	++	+	+	+	+
35	+	-	-	-	-
36	+	-	+	+	+
37	++	+	+	+	+
38	++	-	+	+	+
39	-	-	-	+	+
40	+	-	+	+	+

41	-	-	-	-	-
42	+	-	+	+	+
43	+	+	+	+	+
44	+	+	+	+	+
45	++	++	+	+	+
46	++	++	+	+	+
47	-	-	-	-	-
48	-	-	-	-	-
49	-	-	-	-	-
50	-	-	-	+	-
51	++	++	+	+	+
52	+	+	+	+	+
53	++	+	+	+	+
54	++	++	+	+	+
55	-	-	-	-	-
56	+	+	+	+	+
57	+	+	+	+	+
58	+	-	+	+	+
59	++	++	+	+	+
60	-	-	-	-	-
61	++	++	+	+	+
62	++	+	+	+	+
63	++	++	+	+	+
64	-	-	-	-	-
65	++	++	+	+	+
66	++	+	+	+	+
67	-	-	+	+	+
68	+	+	+	+	+
69	++	++	+	+	+
70	+	-	+	+	+
71	+	-	+	+	+
72	++	++	+	+	+
73	-	-	-	-	-
74	-	-	-	-	-
75	+	-	+	+	+
76	++	++	+	+	+
77	+	+	+	+	+
78	++	+	+	+	+
79	+	-	+	+	+
80	-	-	-	-	-
81	-	-	-	-	-
82	+++	+	+	+	+
83	++	++	+	+	+
84	+	++	+	+	+
85	++	+	+	+	+
86	-	-	-	-	-
87	++	++	+	+	+
88	++	+	+	+	+
89	-	-	-	-	-
90	++	+	+	+	+
91	-	-	-	+	-
92	++	++	+	+	+
93	+	+	+	+	+

(+) Positive, (++) Strongly Positive, (-) Negative

*Microscopy results;

100 microscope scanned area:
no ARB (-), 1-9 ARB (+), 10-99 ARB (++)²⁷

**Culture media (LJ) results;

50-100 CFU (1+), 100-200 CFU (2+)²⁸



Table 4. Analysis Results of Sputum Samples in Different Techniques

Results	ARB Staining	LJ Culture	Gene Expert	Eiken Loopamp	DNA Hunter
Positive	68	56	63	62	64
Negative	25	25	23	19	22
False Positive	-	-	2	6	2
False Negative	-	12	5	6	5
Total	93	93	93	93	93

According to the ARB staining method of the sputum samples obtained, it was found 68/93 positive, and 25/93 negative. Compared with the ARB results; With the LJ culture, 12 samples were obtained as false negatives. The Geneexpert method detected 5 ARB positive samples as negative. The false negative detection rate of the DNA hunter device was the same as the Gene Expert method. Finally, 6 false positives and 6 false negative samples were detected by the Eiken Loopamp LAMP kit. the DNA hunter device with the in-house LAMP method was successful with only 2 false positives and 5 false negatives results.

Although various articles were published about the detection of pathogenic microorganisms by LAMP assay, the use of the LAMP method is not limited to pathogens, its application in the diagnosis of allergens, GMOs, and cancer was reported. The importance of the LAMP method increased especially during the Covid19 pandemic period. The popularity of LAMP depends on its ability to operate at a constant temperature. For this reason, simple heaters have been developed by researchers for usage in the field. Papadakis et al. developed a real-time quantitative colorimetric LAMP (qcLAMP) device. Their device is 3D-manufactured and operates via an in-house developed smartphone application. The size and weight of this device are (11×10×10 cm;370 g.) The device employed a mini digital camera for monitoring in real-time the transition during colorimetric LAMP amplification. The device's clinical evaluation is demonstrated in cancer mutations-analysis and COVID-19 testing.²⁹ Kaygusuz et al. also developed a device

called DiamonD which is used for GMO detection. The device features are 108 g, 6 × 6 × 3 cm. The physical parts of the device were manufactured by using a 3D printer. In this device, Peltier is used as a heating element, different from our study. The LAMP reaction result was evaluated colorimetrically using HNB.³⁰

Liang et al. developed a handheld, automatic, and detection system-free thermal digital microfluidic (DMF) device for LAMP assay. Droplet manipulation and real-time temperature control systems were integrated into a handheld device³¹ called LampPort that performed detection of *Trypanosoma brucei*, a blood parasite (Table 5). In addition to that Hu et al., studied *Salmonella* contamination on eggs with the LAMP method by using Genie II (OptiGene, UK) instrument. This device is commercially available for LAMP analysis and products can be visualized under UV light.³²

Table 5. Comparison of Isothermal Amplification Devices and LAMP Applications

LAMP applications	Devices	Portable	Monitoring of LAMP results
MTB in sputum	DNA Hunter	Yes	Colorimetric
Cancer mutations-analysis, COVID-19 testing (Papadakis et al., 2022)	qcLAMP	Yes	Colorimetric
GMO in soybean (Kaygusuz et al.,2019)	DaimonDNA	Yes	Colorimetric
<i>Trypanosoma brucei</i> in blood (Liang et al.,2019)	LampPort	Yes	UV light
<i>Salmonella</i> ser. Enteritidis in egg products (Hu et al., 2018)	Genie III OptiGene	Yes	UV light
Zika Virus detection (Song et al., 2016)	Disposable cassette	Yes	Colorimetric
Fecal bacteria analysis in water (Lee et al.,2019)	LAMP PCR device	Yes	Colorimetric

Song et al. reported a simple, easy-to-use, LAMP assay for the detection of the Zika virus. the system has a disposable cassette that carries out all the unit operations from sample introduction to detection. The device reported in this study is different from the other devices shown in Table 5, it can operate independently of electricity, as in our device.³³ Lee et al., studied LAMP assay on a

portable device for the detection of indicator microorganisms in environmental water samples.³⁴

In our device, the ability to work for 90 minutes independently without electricity is provided by a low-power consumption Li-Ion battery. This feature of our device provides important benefits besides being portable for analysis in the field. DNA Hunter was successfully used in our other study on the detection of *Streptococcus* type A (GAS).³⁵

STRENGTH AND LIMITATION

The strength of this work was supported as a research project and developed over a 48-month period. There was no time or financial hardship. The LAMP method used in device tests was completed as a project work package and the results were published in other articles. The weakness of the study is that the article writing phase is delayed and takes a long time after the project is completed.

CONCLUSIONS

MTB LAMP assay with the portable device; It can be used in natural disasters and war situations and/or in places with insufficient laboratory infrastructure. DNA Hunter can also be used as a screening test for those who stay in prisons, immigrants, refugees, asylum seekers, come from other countries with a high incidence of tuberculosis, and the homeless. MTB-LAMP is a simple molecular assay that requires less than one hour to perform and can be analyzed by the naked eye.

Following a review of the latest research, WHO suggests that MTB-LAMP can be used as a replacement for microscopy in the diagnosis of pulmonary MTB in adults with signs and symptoms of tuberculosis.

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CONFLICT OF INTEREST

The author declare that she has no conflict of interest.

AUTHOR CONTRIBUTION

As a project coordinator and researcher, I carried out the relevant studies and completed the article writing.

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