International Journal of Technology 12(6) 1207-1216 (2021)
Received August 2021 / Revised October 2021 / Accepted November 2021

International Journal of Technology

http://ijtech.eng.ui.ac.id

Assessment of Cost-Efficient Thermocycler Prototype for Polymerase Chain Reaction and Loop-Mediated Isothermal Amplification

Kenny Lischer[1*], Forbes Avila[1], Muhamad Sahlan[1], Yudan Whulanza[2]

[1]*Department of Chemical Engineering, Faculty of Engineering, Universitas Indonesia, Kampus UI Depok, Depok 16424, Indonesia*
[2]*Department of Mechanical Engineering, Faculty of Engineering, Universitas Indonesia, Kampus UI Depok, Depok 16424, Indonesia*

Abstract. DNA amplification-based diagnostic is the most accurate method among others, especially during the COVID-19 pandemic. Thus, increasing the global demand for instrumentation and amplification reagents locally, hence increasing import. It is a worrying state in terms of logistics and the future domestic market. An effort for domestic production is a must. Previously a cost-efficient thermocycler prototype using Raspberry Pi and Phyton coding is constructed. Thermocycler prototype flow measurement and heat distribution have previously been tested. This research aims to compare thermocycler prototypes and commercial for in two types of DNA amplification reactions, polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP). PCR is the most and more common method than LAMP, with the main difference of PCR require thermal cycling and LAMP operate in isothermal conditions. LAMP has a quicker reaction time and operates at a lower temperature. DNA pol with high strand displacement activity is used for LAMP, in this research Bsm pol is used for LAMP and Taq pol for PCR. Since the prototype thermocycler is designed to be as simple and inexpensive as possible for ease of manufacture and accessibility for every layer of society. Hence, its heat control and stability are not as good as a commercial thermocycler, with huge temperature fluctuation resonance from its set-point. That causes prototype incapability of performing PCR, no DNA band at 250-500 bp range in gel electrophoresis. However, the prototype is capable of performing LAMP, existing <100 bp DNA gradient band in gel electrophoresis. The prototype is also capable of performing LAMP below its protocol temperature and time separately, 62°C and 40 minutes compared to the protocol of 66°C and 60 minutes.

Keywords: DNA amplification-based diagnostic; Loop-Mediated Isothermal Amplification (LAMP); Polymerase Chain Reaction (PCR); Thermocycler prototype

1. Introduction

Indonesia's reliance on the import of thermocycler, among other components, will neglect the total demand domestically and increase the prices. Both local thermocycler prototype is the first step needed for Indonesia to be self-sufficient in its domestic market, regarding DNA amplification technology. Currently, all thermocyclers are still imported from overseas, although many importers are domestic corporations. Indonesia has not tried to develop its own thermocycler.

*Corresponding author's email: lischer.kenny@ui.ac.id, Tel.: +62-87-888017811, Fax.: +62-21-7863515
doi: 10.14716/ijtech.v12i6.5207

However, it has produced its reagent (BioCov-19), yet still imported its components (Tunjung et al., 2020). Additionally, comprehensive knowledge and skills in developing devices related to molecular detection shall benefit other biotechnology fields in the future such as a mapping of Indonesian biodiversity and natural resources (Berawi, 2019).

Polymerase chain reaction (PCR) is the most common and popular DNA amplification diagnostic method (Mullis and Faloona, 1987). However, there are other alternative methods such as loop-mediated isothermal amplification (LAMP) (Notomi et al., 2000; Nagamine et al., 2002; Tomita et al., 2008). The main difference (Table 1) is the temperature requirement throughout the amplification process, PCR requires thermal cycling (denaturation, annealing, and elongation) and LAMP operates at constant temperature (isothermal). Thus, resulting in different instrumentation requirements, thermal cycling demand a higher specification of thermal control compare to the isothermal process. Other differences are the number of primer sets and DNA pol. PCR only uses one set of primer meanwhile, LAMP could use either 2 or 3 sets of primer. DNA pol for isothermal amplification should have high strand displacement activity in its helicase sub-unit. DNA pol from genus bacillus/bacillus has high strain displacement activity and is commercially used in the isothermal reactions, for example, Bst pol from *Bacillus stearothermophilus* (Li et al., 2017; Lischer et al., 2020).

Table 1 Comparison of PCR and LAMP

Aspect	PCR	LAMP
Temperature	Dynamic (thermalcycling)	Constant (isothermal)
Duration	Longer (more than 1 hour)	Faster (~1 hour)
Accuracy	More accurate and consistent	Less accurate and consistent
Equipment requirement	More complex (expensive)	Simpler (cheaper)

Previously an Indonesian-made lab-on-a-chip (LoC) thermocycler prototype is designed and fabricated with a conventional method to achieve the lowest production cost by using existing fabrication process. Plus, the prototype uses readily available material in Indonesia without any import. However, the thermocycler prototype has never been tested for DNA amplification only its flow measurement and heat distribution, resulting in a 10% temperature deviation from its input (Whulanza et al., 2017). This research aims to compare the thermocycler prototype with a commercial thermocycler in both PCR and LAMP reactions, with variations of reaction temperature and time.

2. Methods

2.1. Sample Preparation

The containers, nutrition medium, and micropipette-tips are sterilized using an autoclave (Tomy, Japan) at 121°C for 15 minutes. Water used for DNA amplification is water for injection, purified using an ultra-clear water purifier (Evoqua, USA).

2.2. DNA Purification

E. coli DH5α (Thermofisher Scientific, USA) was cultured overnight in 1 L lysogeny broth (Merck, Germany) at 37°C at an incubator shaker (Ovan, Spain). DNA extraction uses Exgene Clinical DNA Extraction kit (GeneAll, South Korea) and centrifuged (Gyrozen, South Korea). Then the DNA extract is measured using nanodrop (Thermofisher Scientific, USA). DNA extract is considered to be pure if the ratio of absorbance at 260 nm over at 280 nm (A_{260}/A_{280}) is 1.8 (Sambrook, 2001).

2.3. Thermocycler

Thermocycler prototype construction is mainly from acrylic and its heating element is made with a peltier, usually used for water dispensers. Using a Raspberry Pi with Phyton coding as its heat input, control, and temperature recorder. The thermocycler prototype is compared in PCR and LAMP reaction with commercial thermocycler AllInOneCycler™ (Bioneer, Korea) (Figure 1). Because the prototype does not have a heated lid like the commercial, an addition of 30 μL pharmaceutical grade mineral oil (Bartoline, UK) is used to prevent sample evaporation.

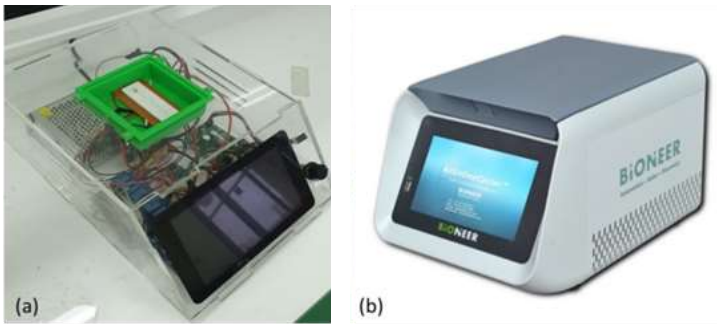

Figure 1 Thermoccyler used in this research: (a) Prototype; (b) Commercial

2.4. Polymerase Chain Reaction (PCR)

Detection of apoptin gene was plasmid pET9a is used as the target for PCR reaction. Using primers (Integrated DNA Technologies, Singapore) ApoF (forward) 5'-CGAAGGGAGGTGTCATAAATGAACGCTCTCCAAGAAGATACTC-3' and ApoR (reverse) 5'-GATGGTGATGGTGATGCGATCCCAGTCTTATACACCTTCTTGCGG-3'. With a total reaction volume of 20 μL per sample. PCR is conducted at 25 cycles with denaturation at 94^{o}C for 30 seconds, annealing at 55^{o}C for 30 seconds, and extension at 72^{o}C for 1 minute (Sahlan et al., 2016). Taq master mix (Smobio, Taiwan) was used for PCR reaction. The composition for PCR can be seen in Table 2.

Table 2 PCR reaction components

Component	Forward primer	Reverse primer	DNA template	Taq Master Mix	WFI
Volume (μL)	1	1	2	10	6

2.5. Loop-Mediated Isothermal Amplification (LAMP)

Detection of *E. coli* malB gene used as the target for LAMP reaction. Using primers (Integrated DNA Technologies, Singapore) F3 5'- GCCATCTCCTGATGACGC-3'; B3 5'-ATTTACCGCAGCCAGACG-3'; FIP 5'-CTGGGGCGAGGTCGTGGTATTCCGACAAACACCACGAATT-3'; and BIP 5'-CATTTTGCAGCTGTACGCTCGCAGCCCATCATGAATGTTGCT-3'. With a total reaction volume of 25 μL per sample. LAMP was conducted at 66^{o}C for 1 hour (Ramezani et al., 2018). Bsm pol (Thermofisher Scientific, USA), Bsm buffer (Thermofisher Scientific, USA), deoxynucleotides (Thermofisher Scientific, USA), and $MgCl_2$ (Merck, Germany) was used for LAMP reaction. The composition for LAMP can be seen in Table 3.

Table 3 LAMP Reaction Components

Component	F3 primer	B3 primer	FIP primer	BIP primer	DNA template	Bsm pol	Bsm buffer	$MgCl_2$	dNTPs	WFI
Volume(μL)	0.5	0.5	1	1	2	1	2.5	4	3.5	9

2.6. Gel Electrophoresis

The gel was made with 2% agarose (Vivantis, Malaysia) in 1X tris-acetate-EDTA (TAE) (Himedia, Indonesia) and addition of NEXview nucleic acid stain (Nex Diagnostic, Indonesia). The samples are loaded into each well of the gel with an addition of 1 μL loading dye (Smobio, Taiwan). Then set the horizontal gel electrophoresis (Indolab Utama, Indonesia) at 100 V for 25 minutes.

0.25-10 kb DNA ladder (Smobio, Taiwan) used for PCR, positive result show amplification in 250-500 bp region (Sahlan et al., 2016). 100 bp DNA ladder (Bioron, Germany) used for LAMP, positive result show amplification below 100 bp (Ramezani et al., 2018). The gel images are processed with ImageJ version 1.53e from RGB to 8-bit format then adjust the contrast and brightness (Schneider et al., 2012).

3. Results and Discussion

Comparison between gel electrophoresis, temperature profile, and DNA concentration measurement with nanodrop is compared between thermocycler prototype and commercial. Reaction temperature and duration are varied from the protocol, to see the robustness of the thermocycler.

3.1. Assessment of PCR

The simple temperature control, lack of cooling, of thermocycler prototype (10 hours and 7 minutes) causes the 25 cycles PCR reaction much longer than commercial (2 hours and 30 minutes). The simplicity of its construction, heat control, and stability, causes a longer reaction time likely to cause Taq pol to denatured prematurely. The heat instability likely causes an unideal and unstable condition for: DNA denaturation; binding of primers to DNA target; and Taq pol elongation at 72^{o}C. Most likely, the problem lies in the denaturation step because in the gel there is no trace of amplification at all. Signifying that the error occurred since the first cycle. The prototype also does not have an initial denaturation and final elongation may contribute to its incapability to perform PCR.

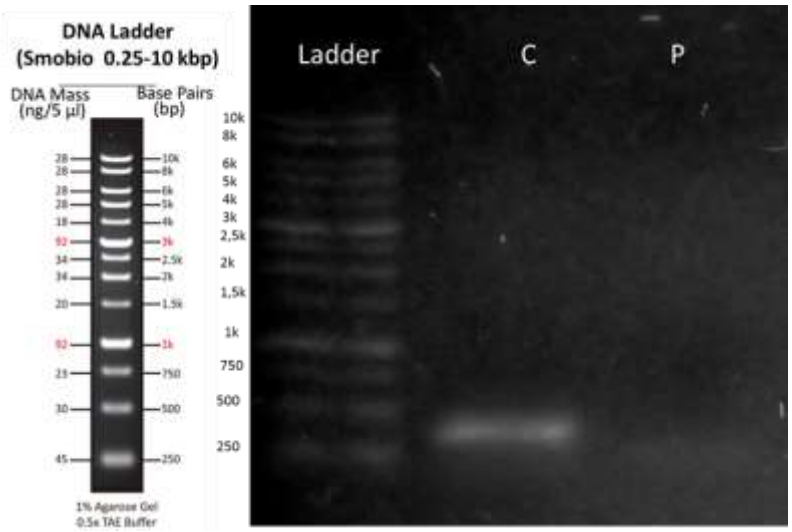

Figure 2 PCR gel comparison (C) commercial bioneer AllInOneCycler (P) prototype

Thus, the thermocycler prototype failed to conduct a PCR reaction, as shown in its gel electrophoresis result (Figure 2), further troubleshooting is needed. Either by modifying its coding (using other models, like PID control) or hardware, with the focus of its heat control

and stability. We compared this research to similar other thermocycler prototype manufacture in academic settings (not the industrial sector), as seen in Table 4.

Previous research builds a cost-effective and simple thermocycler prototype using a Printed Circuit Boards (PCBs) and able to perform conventional PCR, simpler than the thermocycler prototype in this research (Zou et al., 2002). Although it is claimed to be successful in conducting PCR, the DNA band form in its gel electrophoresis is faint and unclear, without comparison with a commercial or prover thermocycler. It seems that reacting the samples in direct contact is better than indirect contact (microtubes). The shorten PCR time (denaturation, annealing, and elongation) is a better approach than copying the same PCR protocol using a commercial or proven thermocycler.

Previous research builds a cost-effective and portable thermocycler prototype using 3D printing and is successful in RT-qPCR, more complex than the thermocycler prototype in this research (Mendoza-Gallegos et al., 2018). The 3D-printed thermocycler prototype has the same objective as the thermocycler prototype used in this study, to be as cost-effective as possible. 3D printing is quite rare and readily available across Indonesia, thus having a higher production cost in Indonesia. However, using 3D printing has its benefit like a smaller form factor and will provide more options of form factor and material choice. However, the 3D-printed thermocycler prototype was designed to perform RT-qPCR with 2 μL well volume compare to this research thermocycler prototype with up to 1 mL microtube and was design to perform conventional PCR. RT-qPCR has a faster reaction time compared to conventional PCR.

Previous research builds a field-deployable and low-cost (FLC) with a much simpler construction (Ferguson et al., 2020) and control unit compare to (Mendoza-Gallegos et al., 2018). The only construction is a pole in the side of components such as peltier, heating block, fan, and control unit to stabilize those components. It can be concluded that newer research does not mean better techonology (3D printing) and advanced components (control unit). These findings suggest that the goals and end specification of the thermocycler prototype are more important than the technology and components, either than the successful result.

Table 4 Comparison of thermocycler protoype research

Comparison	Thermocycler Porotype			
	Micro-Assembly	3D Printed	FLC	This Research
Construction	Micro-Assembly	3D-printed	No outer body	Manual labour
Control	Printed Circuit Board (PCB)	Raspberry Pi & Phyton	ATMEGA328P-PU & C++	Raspberry Pi & Phyton
Well volume	20 μL	2 μL	Unspecified	Up to 1 mL
PCR Method	Conventional PCR	RT-qPCR	Conventional PCR	Conventional PCR
Cycle	30	40	35	25
Total duration	15 minutes	~2 hours	Unspecified	10 hours and 7 minutes
Result	Successful (faint DNA band)	Successful	Successful	Unsuccessful
Citation	Zou et al., 2002	Mendoza-Gallegos et al., 2018	Ferguson et al., 2020	Whulanza et al., 2017

3.2. Assessment of LAMP

Both LAMP reactions by thermocycler prototype and commercial are conducted at the same time because LAMP does not depend on cycle. Because of thermocycler prototype heat instability, a lower temperature rection is tested, at 62°C. According to the protocol, the reaction temperature is 66°C (Ramezani et al., 2018).

The thermocycler prototype successfully performs LAMP at 62°C (P62) and 66°C (P66). Meanwhile, commercial thermocycler LAMP was only successful at 66°C (C66) and failed at 62°C (C62). The DNA band generated from the thermocycler prototype are thicker than commercial thermocycler, suggesting more DNA amplification. Meaning that although the thermocycler prototype lacking in heat control and stability, it is a more robust thermocycler than a commercial thermocycler. Hence, the thermocycler prototype is able to perform LAMP at a lower temperature than commercial. From an energy consumption perspective, the thermocycler prototype is better than the commercial, while still able to performs LAMP. Which fits the design purpose of the thermocycler prototype for remote areas. Where an electrical source may be scarce.

Gel electrophoresis data are aligned with nanodrop measurement that thermocycler prototype LAMP amplification is higher than commercial thermocycler. Even, amplification at P62 is higher than C66, its supposed reaction temperature. Thus, the thermocycler prototype is a more robust instrument in terms of reaction temperature according to gel electrophoresis and DNA measurement with nanodrop, as seen in Figure 3.

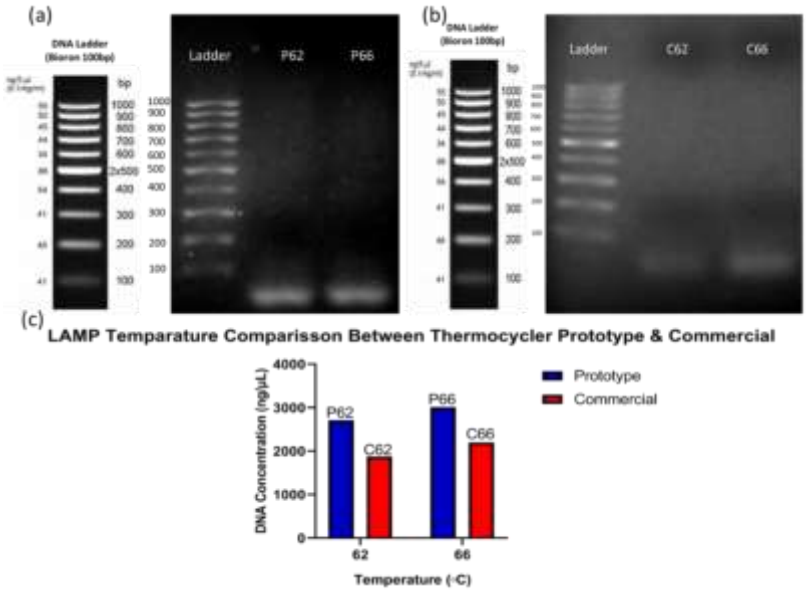

Figure 3 LAMP gel electrophoresis: (a) Thermocycler prototype LAMP result; (b) Commercial AllInOneCyler Bioneer thermocycler LAMP result; (c) LAMP amplicon nanodrop DNA measurement, (P) stands for thermocycler prototype, (C) stands for commercial AllInOneCyler Bioneer thermocycler, (62) LAMP conducted at 62°C for 1 hour, (66) LAMP conducted at 66°C for 1 hour

Then to further test the robustness of the thermocycler prototype, LAMP reaction times are varied from: 40, 60, 80, 100, and 120 minutes at 66°C. According to the protocol, the LAMP duration is 60 minutes (Sahlan et al., 2016).

Once again thermocycler prototype produced a thicker DNA band compare to the commercial thermocycler. The thickest DNA band was found at 60 minutes using a thermocycler prototype (P6). A thermocycler prototype could conduct LAMP with a shorter reaction time than a commercial thermocycler, as seen in the existing band in P4 meanwhile at C4 no band exists, as seen in Figure 4.

DNA concentration measurement with nanodrop shows that the highest concentration of DNA is found at P8, although P6 has a more dense and visible band in gel electrophoresis. However, the general trend is that amplification results are higher with using thermocycler prototype compare to commercial, with an exception at 2 hours.

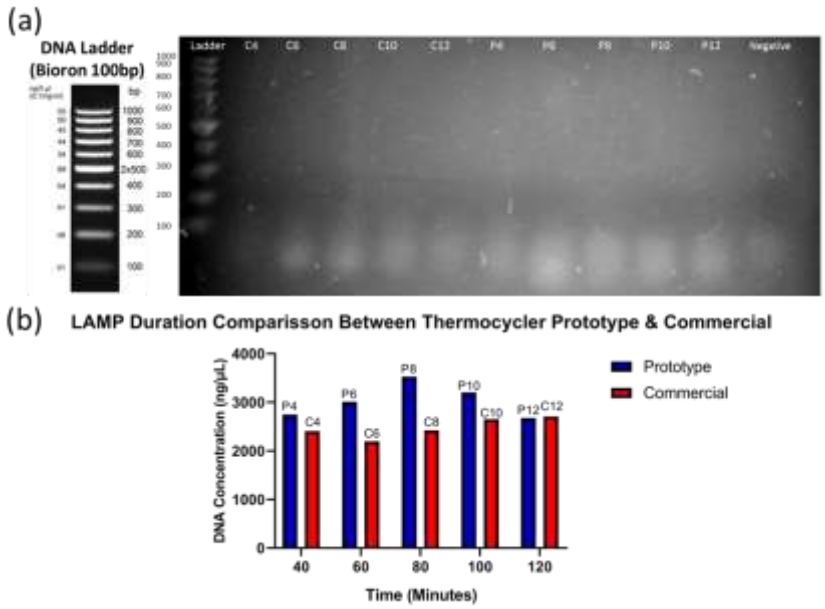

Figure 4 LAMP gel electrophoresis: (a) Thermocycler prototype and commercial AllInOneCyler LAMP result comparison; (b) LAMP amplicon nanodrop DNA measurement, (P) stands for thermocycler prototype, (C) stands for commercial AllInOneCyler Bioneer thermocycler, (4) LAMP conducted at 66°C for 40 minutes, (6) LAMP conducted at 66°C for 60 minutes, (8) LAMP conducted at 66°C for 80 minutes, (10) LAMP conducted at 66°C for 100 minutes, and (12) LAMP conducted at 66°C for 120 minutes

Previous research builds a cost-effective and low resource construction thermocycler prototype with a reactor shape is able to perform RT-qLAMP. Due to its simplicity, the simple thermocycler prototype is specifically made for isothermal DNA amplification process, LAMP (LaBarre et al., 2010). Its performance, amplicon fluorescent, could match Perkin-Elmer GeneAmp9600 (Artisan Technology Group, Tunisia) a commercial thermocycler. Which fits the case for this research that a simple thermocycler prototype could perform LAMP equally if not better compare to a commercial thermocycler.

Surprisingly many thermocycler prototypes are tested in PCR, instead of LAMP. Even though LAMP's thermocycler requirement is simpler than PCR. The reasoning behind that may be that PCR is a more common method, PCR has better sensitivity, and/or that by being able to perform PCR (more complex) the thermocycler is able to perform in a simpler method like LAMP.

3.3. Temperature Profile of Thermocycler

The thermocycler prototype has a function to record the temperature in its heating block. The recorded heat is converted into a graph and compared with the commercial thermocycler. The offset and resonance from the prototype cause the real-time to exceed the input time. For example, 25 cycle PCR using thermocycler commercial takes 10 hours and 7 minutes, instead of 2 hours and 30 minutes with commercial thermocycler.

The longer period in PCR may cause the DNA pol to denature along the PCR process. Its instability is clearly shown in the elongation step more than in any other stages of PCR. Elongation takes 1 minute instead of 30 seconds in denaturation and annealing. It has not been tested to operate in real-time, in the process ignoring the coding and heat instability, as seen in Figure 5.

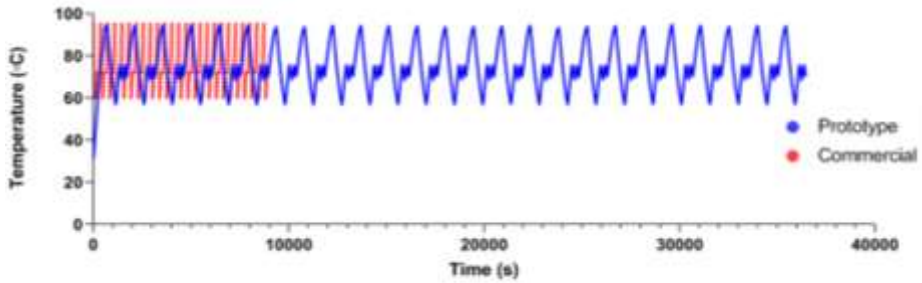

Figure 5 Temperature profile of PCR process with prototype & commercial thermocycler

The temperature profile of LAMP at P62 and P66 intersect at the positive offset of P62 and the negative offset of P66. Thus, the temperature profile with 62 and 66°C input is roughly the same. Probably the cause of LAMP with thermocycler prototype is successful in both 62 and 66°C. Meanwhile, commercial thermocycler only successful at 66°C and failed at 62°C. This phenomenon could be because of its heat control and stability, as seen in Figure 6.

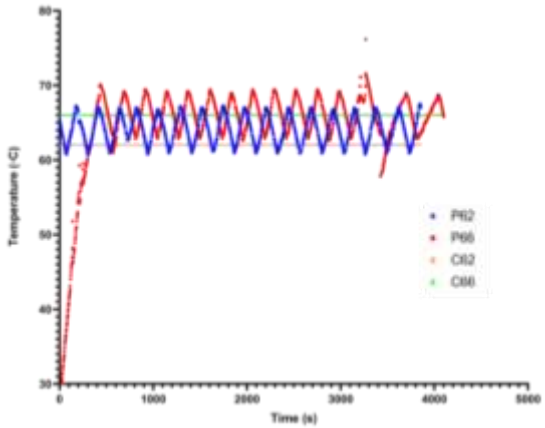

Figure 6 Temperature profile of LAMP process with prototype & commercial thermocycler

Micro-assembly and 3D printed thermocycler prototype both have better heat control and stability, thus successfully conduct PCR, it has a precision of ±0.1°C and ±0.42°C respectively (Zou et al., 2002; Mendoza-Gallegos et al., 2018). Meanwhile, the thermocycler prototype used in this research has a 10% temperature deviation (Whulanza et al., 2017).

4. Conclusions

The thermocycler prototype heat control and stability compare to other prototypes and commercial thermocyclers. Thus, it could not perform a conventional PCR reaction. To tackle this problem the PCR duration (denaturation, annealing, and elongation) could be shortened, decreasing the reaction volume, and direct contact between sample and heating element of thermocycler (without microtube). The coding parameter and addition of better components could also increase its heat control and stability. However, the lack of heat control and stability does not hinder the thermocycler prototype's ability to perform LAMP. Due to its lack of heat control and stability, it becomes a more robust thermocycler compare to thermocycler commercial. It is able to perform LAMP at a lower temperature (62°C instead of 66°C) and in a shorter period (40 minutes instead of 60 minutes), separately. Future research needs to develop the prototype on its construction and coding to a more advanced prototype, that is eventually suitable for mass production.

Acknowledgements

The authors are grateful for the grant PUPT grant NKB 216/UN2.RST/HKP.05.00/2021 for finding this research. In addition, KL and YW as research supervisors and made the research concept. FA as researcher and writer, and MS as research supervisor.

References

Berawi, M.A., 2019. Managing Nature 5.0 in Industrial Revolution 4.0 and Society 5.0 Era. *International Journal of Technology*, Volume 10(2), pp. 222–225

Ferguson, J., Duran, J., Killinen, W., Wagner, J., Kulesza, C., Chatterley, C., Li, Y., 2020. A Field-Deployable and Low-Cost PCR (FLC-PCR) Thermocycler for the Rapid Detection of Environmental E. coli. *In:* Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS, 2020-July, 2209–2212

LaBarre, P., Gerlach, J., Wilmoth, J., Beddoe, A., Singleton, J., Weigl, B., 2010. Non-Instrumented Nucleic Acid Amplification (NINA): Instrument-free Molecular Malaria Diagnostics for Low-Resource Settings. *In:* 2010 Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBC'10, 1097–1099

Li, P., Amenov, A., Kalendar, R., Abeldenov, S., Khassenov, B., 2017. Cloning and Purification of Large Fragment of DNA Polymerase I from Geobacillus Stearothermophilus and it's Application in Isothermal DNA Amplification. *Biotechnology. Theory and Practice*, *March 2017*

Lischer, K., Tansil, K.P., Ginting, M.J., Sahlan, M., Wijanarko, A., Yohda, M., 2020. Cloning of DNA Polymerase I Geobacillus thermoleovorans SGAir0734 from a Batu Kuwung Hot Spring in Escherichia coli. *International Journal of Technology*, Volume 11(5), pp. 921–930

Mendoza-Gallegos, R.A., Rios, A., Garcia-Cordero, J.L., 2018. An Affordable and Portable Thermocycler for Real-Time PCR Made of 3D-Printed Parts and Off-the-Shelf Electronics. *Analytical Chemistry*, Volume 90(9), pp. 5563–5568

Mullis, K.B., Faloona, F.A., 1987. Specific Synthesis of DNA in Vitro via a Polymerase-Catalyzed Chain Reaction. *Methods in Enzymology*, Volume 155(C), pp. 335–350

Nagamine, K., Hase, T., Notomi, T., 2002. Accelerated Reaction by Loop-Mediated Isothermal Amplification using Loop Primers. *Molecular and Cellular Probes*, Volume 16(3), pp. 223–229

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., Hase, T., 2000. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, Volume 28(12), https://doi.org/10.1093/nar/28.12.e63

Ramezani, R., Parizi, Z.K., Ghorbanmehr, N., Mirshafiee, H., 2018. Rapid and Simple Detection of Escherichia Coli by Loop-Mediated Isothermal Amplification Assay in Urine Specimens. *Avicenna Journal of Medical Biotechnology*, Volume 10(4), pp. 269–272

Sahlan, M., Bela, B., Bowolaksono, A., Malik, A., Yohda, M., 2016. The Expression and Purification of Octa-Arginine Apoptin and its Ability to Kill Cancer Cells. *International Journal of Pharmacy and Pharmaceutical Sciences*, Volume 8(10), pp. 102–104

Sambrook, J., 2001. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 Years of Image Analysis. *Nature Methods*, Volume 9(7), pp. 671–675

Tomita, N., Mori, Y., Kanda, H., Notomi, T., 2008. Loop-Mediated Isothermal Amplification (LAMP) of Gene Sequences and Simple Visual Detection of Products. *Nature Protocols*,

Volume 3(5), pp. 877–882

Tunjung, N., Kreshanti, P., Saharman, Y.R., Whulanza, Y., Supriadi, S., Chalid, M., Anggraeni, M.I., Hamid, A.R.A.H., Sukasah, C. L., 2020. Clinical Evaluation of Locally Made Flocked Swabs in Response to the COVID-19 Pandemic in a Developing Country. *International Journal of Technology*, Volume 11(5), pp. 878–887

Whulanza, Y., Aditya, R., Arvialido, R., Utomo, M.S., Bachtiar, B.M., 2017. Ease Fabrication of PCR Modular Chip for Portable DNA Detection Kit. *In:* AIP Conference Proceedings, 1817(1), https://doi.org/10.1063/1.4976791

Zou, Q., Miao, Y., Chen, Y., Sridhar, U., Chong, C.S., Chai, T., Tie, Y., Teh, C.H. L., Lim, T.M., Heng, C.K., 2002. Micro-Assembled Multi-Chamber Thermal Cycler for Low-Cost Reaction Chip Thermal Multiplexing. *Sensors and Actuators, A: Physical*, Volume 102(1–2), pp. 114–121