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Preface

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Heating and Cooling Rate Study on Low-Power Water Cooling Thermal Cyclers using Aluminium Block Sample

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ABSTRACT Temperature measurement has many applications in medical devices. In recent days, body temperature become the main screening procedure to justify people infected by SARS-CoV-2. Related to pandemic situation due to SARS-Cov-2, Polymerase Chain Reaction (PCR) method become the most accurate and reliable detection method. This method employs a device named PCR machine or Thermal Cycler. Commercially available thermal cycler draws 350-950 Watt from its power source. In this research, we focus to build a Thermal Cycler using a low-cost material such as aluminium, using a liquid coolant as the cooling system, and draw on approximately 180 Watt from the power source. We use 2 types of coolant solution: mineral water and generic liquid coolant. Peltier device in thermal cycler serves as heating and cooling element. In heating rate experiments, generic liquid coolant shows a better result than using mineral water due to specific heat capacity and thermal conductivity of water. In the cooling rate experiments, the water pump is activated to stream the liquid solution, the flow rate of liquid solution is influenced by viscosity of the liquid. Generic liquid coolant has approx. 4,5 times greater viscosity than water. The higher flow rate means better performance for cooling rate. Using 2 pieces of 60-Watt heaters and a 60-Watt chiller and aluminium material as block sample, our research shows a heating and cooling rate up to approx. 0,1°C/s. Compared to commercially thermal cycler, our thermal cycler has a lower wattage; this lower wattage performance has been tradeoff with lower ramping rate. Some factors are suspected become the source of contributors of lower ramping rate.

INDEX TERMS Cooling Rate, Heating Rate, Polymerase Chain Reaction, PCR, Thermal Cycler.

I. INTRODUCTION

Temperature measurement has many applications in medical devices. For example, in recent days, body temperature become the main screening procedure to justify people infected by SARS-CoV-2. To name a few, temperature measurement also become a critical factor in medical device sterilization process, blood warming technology, laboratory water bath, and hot plate stirrer.

Related to pandemic situation due to SARS-Cov-2, Polymerase Chain Reaction (PCR) method become the most accurate and reliable detection method. This method was developed by Karry Mullis in 1984 [1] and widely used in biochemical and molecular biology laboratories [2]–[5]. This method employs a device named PCR machine or Thermal

Cycler. Temperature measurement technology is used in this device as a temperature reference point for its 3 steps. PCR steps are Denaturation, Annealing, and Extension. Temperature sensor is placed in a metal material functioned as block sample for PCR tube. According to World Health Organization Regional Office for South-East Asia [6], some of important specifications for Thermal Cycler are cooling rate of block sample (up to 2°C/sec), heating rate of block sample (up to 3°C/sec), block sample temperature range (4°C - 99°C), and block sample uniformity.

In a low-resource setting, the availability of material types and machining process for block sample become the main issue. We want to address this issue by selecting most common metal as the block sample, aluminium. Aluminium also the

most common material that is used as a block sample in Thermal Cycler [7]. Sailaja and Raju [7] and Mirales, et al. [8] mentioned that Peltier device is the most common component that is used as heating and cooling system. Leading manufacturer in Thermal Cycler, such as Applied Biosystems (Model: ABI Prism 7000 and ABI Prism 7900 HT), Stratagene (Model: Mx4000 and Mx3000P), BioRad (Model: iCycler IQ), and MJ Research (Model: DNA Engine Opticon2), also use Peltier device as the heating and cooling systems [9].

Peltier device as a cooling system uses air as a coolant, but due to thermal conductivity of air, the heat cannot be completely removed ($<10^6$ W/m²) from the device. Liquid cooling on the other hand, show a promising solution to this problem. Alihosseini [10] study the importance of liquid cooling as a cooling system in Thermal Cycler using numerical simulation.

The archaic method –automated hand transfer or dip and dunk method– is claimed has a rapid solution [11] but it uses only 3 set point and 3 container which is not good enough because some PCR Cycling sometimes need 4 set points of different temperature [12] and the initial setting for the thermos water bath [11] is complicated need to be determined empirically using certain estimation formula.

TABLE 1

Comparison of Wattage and Ramping Rate from Several Commercially Available Thermal Cycler

Company & Model	Wattage (Watt)	Ramping Rate	
		Heating Rate (°C/s)	Cooling Rate (°C/s)
Bio-Rad S1000 [13]	700*	2 - 3,3**	
Bio-Rad Opticon 2 [14]	850*	3*	
Eppendorf Mastercycler Pro 384 [15]	950*	4***	3***
Eppendorf Nexus (Gradient & Eco) [15]	700*	3***	2***
Chai Biotech. Open qPCR [16]	350*	5*	
Cepheid SmartCycler II [17]	350	10*	2,5*

* Up to/Max, ** Average, *** ca.

Most commercial product draw a high wattage (from 350-Watt up to 950 Watt) to achieve a fast-ramping rate as shown in Table 1. In their product specification sheet, Eppendorf also uses aluminium for the sample block. Cepheid Smart Cycler II achieve high heating rate up to 10°C/s because they define their ramping rate based on 50°C - 95°C temperature measurement. The other manufacture, except Bio-Rad S1000, mention their ramping rate based on the maximum value that can be achieved by the heating/cooling element.

In this research, we focus to build a Thermal Cycler using a low-cost material such as aluminium and using a liquid coolant as the cooling system. We use 2 types of coolant solution: mineral water and generic liquid coolant. The method and result of this research will be described in the next section.

II. METHOD

Figure 1 shows a simplified block diagram of Thermal Cycler [18]. Peltier device in the system usually serves as heating and

cooling element. Pamungkas [19], Poernomo [20], and Atmani [21] also developed a thermal cycler using Peltier device and aluminium as block sample based on this simplified block diagram. Their thermal cycler [19]–[21], only achieved 0,3 – 0,6 °C/s due to the design of block sample and uniformity of the aluminium materials.

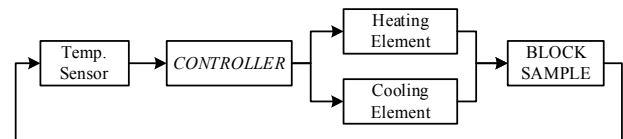


FIGURE 1. Simplified block diagram of Thermal Cycler

Using a liquid as a cooling solution, we need to modify the simplified block diagram become the block diagram as shown in Figure 2. Water reservoir is used to store the coolant solution. As mentioned before, we use mineral water and generic liquid coolant. Generic liquid coolant is usually made of 50% of water with 50% of ethylene glycol (C₂H₆O₂). The difference between mineral water and generic liquid coolant lies on its properties. Stone [22] and Jack [23] summarize the fluid's properties of water and generic liquid coolant as shown in Table 2. Compared to aluminium with its specific heat capacity's properties of 0,9 kJ/kg·K, water and generic liquid coolant draw more energy than aluminium.

TABLE 2

Properties of Water and Generic Liquid Coolant

Properties	Water	Ethylene Glycol/Water (1:1)
Boiling point, 1 bar (°C)	100	111
Freezing Point (°C)	0	-37
Enthalpy of vaporization (MJ/kmol)	44	41,2
Specific heat capacity (kJ/kg·K)	4,25	3,74
Thermal conductivity (W/m·K)	0,69	0,47
Density 20°C (kg/m ³)	998	1057
Viscosity 20°C (cS, 10 ⁻⁶ m ² /s)	0,89	4

Figure 3 and Figure 4 show the proposed design based on the block diagram on Figure 2. We use thermocouples as temperature sensor that was inserted in the block sample. Measured block sample's temperature is the average of thermocouples measurements. Type-K thermocouples are employed as a sensing element in this system and MAX31855 is integrated in the system as the signal conditioning circuits. MAX31855 is an integrated circuit which has an ability to convert analog signal to digital with 14-bit resolution and to compensating the missing thermoelectric voltage due to one of the cold-junction on the one end of thermocouple is not at 0°C. Using virtual reference of 0°C, there is voltage changes approximately 41μV for a 1°C temperature reading in type-K thermocouple. This approximation then calculated using equation (1). Where V_{OUT} is output voltage of type-K thermocouple in μV, temperature of another end of cold-

junction (remote-junction) is T_R (in $^{\circ}\text{C}$), and device temperature is T_{AMB} (in $^{\circ}\text{C}$) [24].

$$V_{OUT} = (41,276 \mu\text{V} / ^{\circ}\text{C}) \times (T_R - T_{AMB}) \quad (1)$$

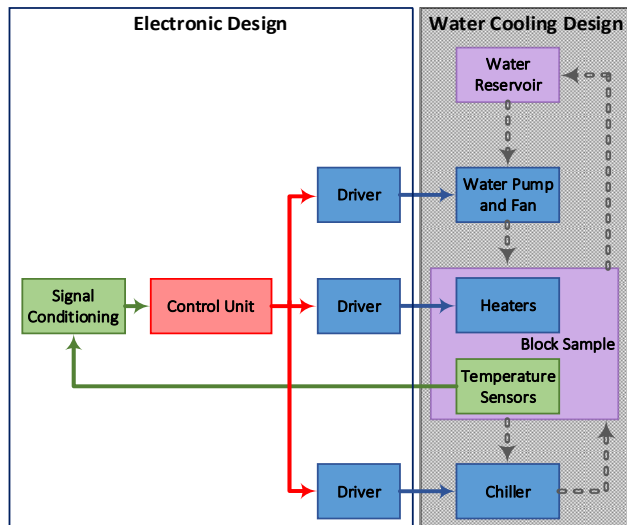


FIGURE 2. Water cooling thermal cycler.

Size of block sample are $80 \text{ mm} \times 80 \text{ mm} \times 16 \text{ mm}$ and can handle up to 3 PCR tubes 1,5 ml with 11 mm diameters. A 60-Watt Peltier device is placed in the chiller to cool the water solution. Coolant solution is pumped using water pump from water reservoir to block sample and back to the water reservoir. To heat the block sample, we use 2 pieces of 60-Watt heating elements which are placed under the block sample.

We design the experiment to run on 35°C up to 75°C for the heating ramp and 75°C up to 35°C for cooling ramp. The lowest temperature is used to study the correlation between room temperature with heating and cooling rate. There are 2 variance of experiments that we design, (1) the chiller condition and (2) types of liquid coolant. From those experiments, we want to investigate the ramping rate ($^{\circ}\text{C/s}$) and the time duration to achieve desired temperature setting (minutes:second). The ramping rate of thermal cycler defines the ability of thermal cycler to ramp between initial temperature to desired temperature and the total time needed to complete 30 cycles of Denaturation, Annealing, and Extension. Ramping rate become the key consideration to determine the performance of thermal cycler [25].

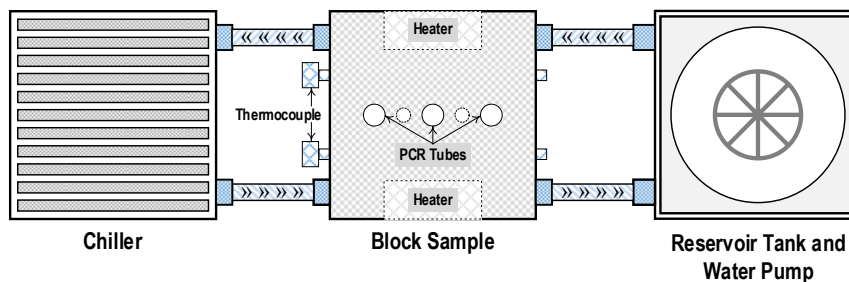


FIGURE 3. Proposed design of water-cooling Thermal Cycler

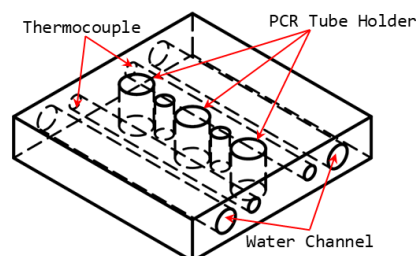


FIGURE 4. Detail and isometric-view of Block Sample

III. RESULT AND DISCUSSION

The result will be explained in three sub-sections: heating rate, cooling rate, and experiments summary.

A. HEATING RATE

We conduct 2 experiments with variation of coolant solution. Each experiment was repeated 5 times (trials). Figure 5 show the heating rate experiment using mineral water (a) and liquid

coolant (b). Figure 6 and Figure 7 show the summary of heating rate duration and heating rate in $^{\circ}\text{C/s}$. Even the purpose of mineral water and generic liquid coolant is for cooling the block sample, but in fact it also affects the duration to heat block sample. It shows that using generic liquid coolant have a better result than using mineral water.

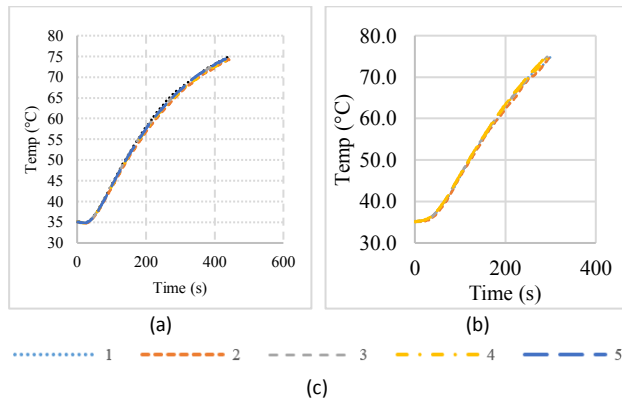


FIGURE 5. Heating rate experiment on (a) Mineral water, (b) Liquid Coolant, and (c) legend

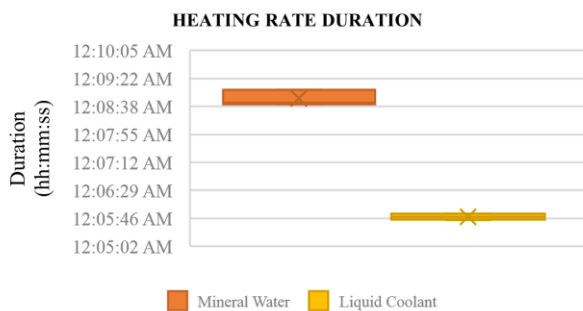


FIGURE 6. Heating rate duration

To achieved $\Delta T = 40^\circ\text{C}$ – from 35°C to 75°C , the system only needs less than 6 minutes. It means that we can achieve up to $0,117^\circ\text{C/s}$. In these 2 experiments, both liquid solutions were not pumped through the tubing; the liquid solution stay still in its place. Based on parameter shown in Table 2, specific heat capacity and thermal conductivity of water greater than generic liquid coolant. Those parameters make the liquid coolant has a better performance than mineral water.

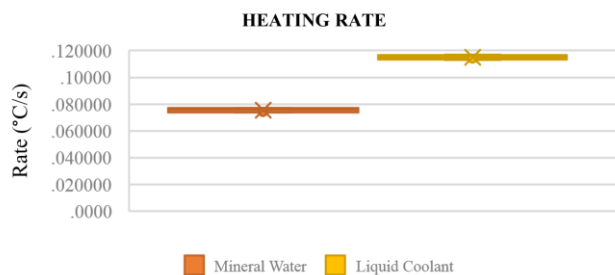


FIGURE 7. Heating rate of proposed water-cooling Thermal Cycler design

B. COOLING RATE

As shown in Figure 8, we conduct 4 experiments. Each experiment was repeated 5 times (trials). The cooling rate duration are shown in Figure 9 and the cooling rate in $^\circ\text{C/s}$ are shown in Figure 10.

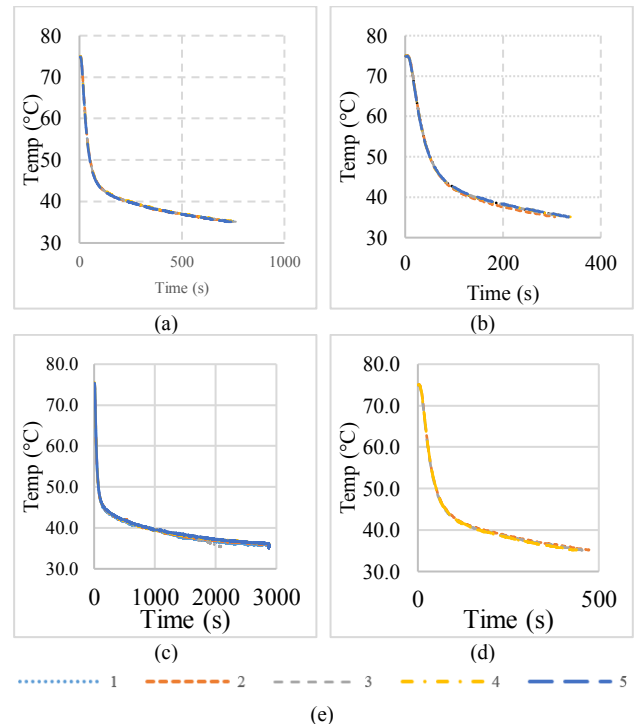


FIGURE 8. Cooling rate experiments on (a) Mineral water with Chiller OFF, (b) Mineral water with Chiller ON, (c) Liquid Coolant with Chiller OFF, (d) Liquid Coolant with Chiller ON, (e) legend

The use of generic liquid coolant without Peltier device is activated increase the time to achieve its set point of 35°C . It takes up 1 hour 50 minutes and 40 seconds to cool down from 75°C to 35°C , it means we can only achieve $0,006^\circ\text{C/s}$. This result shows that the generic liquid coolant is slow down the cooling rate.

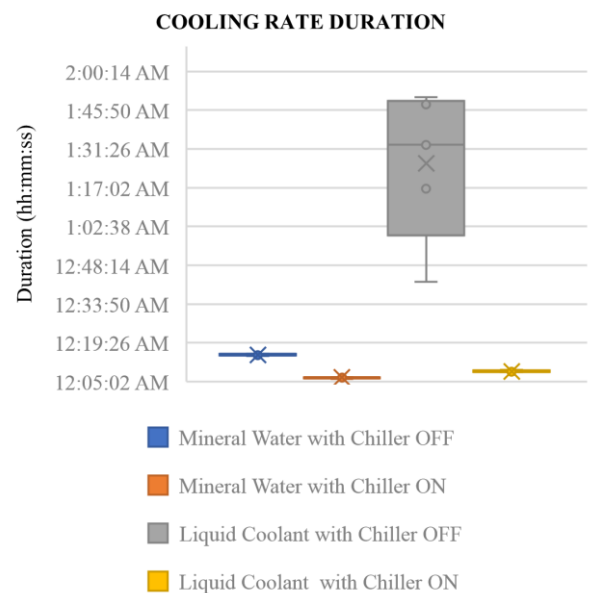


FIGURE 9. Cooling rate duration

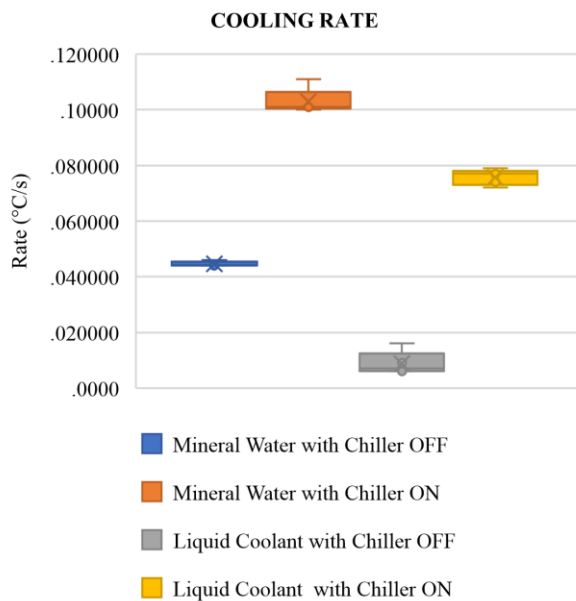


FIGURE 10. Cooling rate of proposed water-cooling Thermal Cycler design

In these 4 experiments, the water pump is activated to stream the liquid solution (water or generic liquid coolant) from the water reservoir to block sample and chiller and loop-back again to water reservoir tank. The flow rate of liquid

solution is influenced by viscosity of the liquid. Compared to the water's viscosity, generic liquid coolant is approx. 4,5 times greater. With the same water pump's power, the flow rate of mineral water is higher than generic liquid coolant. The higher flow rate means better performance for cooling rate.

C. EXPERIMENTS SUMMARY

Summary of heating and cooling rate is shown in Table 3. In heating condition, generic liquid coolant gives a better performance by approx. 52% than mineral water. Overall performance of cooling condition with chiller is activated, mineral water gives a better cooling rate by approx. 36%.

A phenomenon of exponential decay in temperature change occurs in Table 3; there is a temperature inertia in all experiments, especially when temperature measurement reaches approx. 45 °C. This phenomenon also stated in Newton's law of cooling, the rate of heat loss of a body (block sample) is directly proportional to the difference in the temperatures between the body (block sample) and its surroundings.

Due to Newton's law of cooling, we further investigate the rate of cooling with $\Delta T = 30^\circ\text{C}$ – from 75°C to 45°C, the results are shown in Tabel 4. Using this investigation, we can achieve cooling rate from 0,393°C/s up to 0,409°C/s. Mineral water has around 4% better performance than generic liquid coolant.

TABLE 3

Summary of Heating And Cooling Rate With Chiller Condition Is On

Parameter	Trials					Average
	1	2	3	4	5	
Heating Duration	(mm:ss)					
Mineral Water	08:42	09:04	08:42	09:04	08:44	08:51
Liquid Coolant	05:52	05:53	05:44	05:48	05:43	05:48
Heating Rate	(°C/s)					
Mineral Water	0,077	0,074	0,077	0,074	0,076	0,0756
Liquid Coolant	0,114	0,113	0,116	0,115	0,117	0,115
Cooling Duration	(mm:ss)					
Mineral Water	06:37	06:00	06:36	06:39	06:33	06:29
Liquid Coolant	09:17	09:01	08:29	08:37	08:41	08:49
Cooling Rate	(°C/s)					
Mineral Water	0,101	0,111	0,101	0,1	0,102	0,103
Liquid Coolant	0,072	0,074	0,079	0,077	0,077	0,0758

TABLE 4

Further investigation of cooling duration and cooling rate with $\Delta T = 30^\circ\text{C}$

Parameter	Trials					Avg
	1	2	3	4	5	
Cooling Duration	(mm:ss)					
Mineral Water	01:13	01:13	01:14	01:13	01:14	01:13
Liquid Coolant	01:19	01:15	01:15	01:17	01:16	01:16
Cooling Rate	(°C/s)					
Mineral Water	0,411	0,411	0,405	0,411	0,405	0,409
Liquid Coolant	0,380	0,400	0,400	0,390	0,395	0,393

IV. CONCLUSION

Using 2 pieces of 60-Watt heaters and a 60-Watt chiller and aluminium material as block sample, our research shows a heating and cooling rate up to approx. $0,1^{\circ}\text{C/s}$. Compared to commercially thermal cycler, our thermal cycler has a lower wattage; this lower wattage performance has been tradeoff with lower ramping rate. Some factors are suspected become the contributors of lower ramping rate: the determination of lower temperature's set point of 35°C affected by Newton's law of cooling; fluid's properties or characteristics affecting the water pump's power selection and liquid solution's mass; and block sample design that was thick. A further investigation of cooling rate with $\Delta T = 30^{\circ}\text{C}$ – from 75°C to 45°C – shows a promising result of $0,4^{\circ}\text{C/s}$ cooling rate.

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