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Designing Microfluidic PCR Chip Device Using CFD Software for the Detection of Malaria

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Abstract: Polymerase chain reaction (PCR) technique is one of the molecular methods in amplifying DNA for the detection of malaria. However, the collection and transportation of samples and the processing and dissemination of results via conventional PCR, especially when used for routine clinical practice, can hamper the technique's sensitivity and specificity. The rampancy of such disease in the Philippines is aggravated by the limited supply of medical machinery and the poor economic state of the country; thus, the need to innovate a device for the early detection of malaria is necessary. With that, this study focuses on designing a microfluidic device that will mimic the function of a conventional genus-specific PCR based on the 18S rRNA gene to detect malaria parasites (*Plasmodium falciparum*) at low-grade parasitemia. The design was intended to be portable, accessible, and economical, which none from past literature has dealt with specifically for malaria detection. This *in silico* design is a first in the country specially crafted for such reasons. The proposed device was developed and simulated using ANSYS software for Computational Fluid Dynamics (CFD) analyses. The simulation shows that adding loops to the design increases its relative deviation but minimally compared to having only a straight path design. This indicates that looping is acceptable in designing a microfluidic device to minimize chip length. It was also found that increasing the cross-sectional area of the fluid path decreases the efficiency of the design. Lastly, among the three materials utilized, the chip made of polypropylene is the most efficient, with a relative deviation of 0.94 compared to polycarbonate and polydimethylsiloxane, which have relative deviations of 2.78 and 1.92, respectively. Future researchers may mesh the 44-cycle microfluidic chip due to the limitations of the software used in this study, and other materials, such as biocomposites, may be assessed to broaden the application of the design.

Keywords: polymerase chain reaction (PCR); computational fluid dynamics (CFD); *Plasmodium falciparum*; microfluidic chip design

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1. Introduction

Malaria is a disease caused by Plasmodium parasites of different species, such as *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae,* and *Plasmodium ovale.* Among these species, *Plasmodium falciparum* and *Plasmodium vivax* are the most common, and *Plasmodium falciparum* is the deadliest [1]. These parasites are transmitted to people through the bites of infected mosquitoes, and malaria is prevalent in tropical and subtropical countries [2]. In 2021, according to the World Health Organization, an estimated 247 million malaria cases were recorded among the 84 malaria-endemic countries, with the African region accounting for around 95% of the total cases globally [3]. Aside from that, 13.4 million cases between 2019 and 2021 were aggravated by the ramifications of the COVID-19 pandemic [4]. Individuals from continuous transmission areas may, after several malaria infections, develop the premonition state, which is characterized by an immune response

that can control the parasitemia but is unlikely to purge all the circulating parasites [5]. As a result, those individuals can stay asymptomatic and act as a parasitic reservoir since such infected blood can now infect mosquito vectors. With that, these may reintroduce malaria into other regions [6].

The foundation of PCR is the enzymatic amplification of specific DNA sequences. It uses the capacity to synthesize DNA strands of the heat-resistant Taq DNA polymerase [7]. The specific DNA region of the malaria parasite is amplified exponentially, producing millions of copies that are readily detectable. Targeted DNA sections within the parasite's genome are used in PCR to identify malaria parasites. To identify different strains, these areas must be retained across different Plasmodium species [8]. Short DNA sequences called primers are created to selectively attach to the DNA of the parasite and flank the target region [9]. So, to detect malaria with high sensitivity and specificity, primer design is essential. The designed nucleotides, Taq polymerase enzyme, and the extracted DNA are combined. Denaturation, annealing, and extension cycles are repeatedly performed during thermal cycling [10]. Exponential amplification occurs as a result of each cycle doubling the size of the targeted DNA region. Several techniques can be used to determine whether malaria parasite DNA is present following PCR amplification [11]. Gel electrophoresis is a popular technique that uses an electric field to separate the amplified DNA fragments according to size [12]. DNA-staining dyes can be used to see the resultant bands. As an alternative, real-time PCR can be done to analyze DNA amplification quantitatively in real-time [13]. The fluorescence signal for each cycle is evaluated using fluorescent probes or DNA-binding dyes. The amount of DNA amplification coincides with the fluorescence level. PCR has many benefits for finding malaria parasites. It is sensitive and can find even a single parasite DNA molecule in a sample [14].

Additionally, it offers great specificity, reducing false-positive outcomes. PCR can identify drug-resistant strains and distinguish between several Plasmodium species [6]. However, PCR is difficult to use when resources are limited because it calls for specialized lab equipment and skilled technicians [15].

This concept of miniaturizing PCR aims to speed up the process of conventional PCR by reducing the overall sample volume and consumption of reagents, lessening the cost of fabrication, and developing a field-based real-time PCR platform that is capable of completely conducting analyses from raw samples into promising results that will ensure a better method of diagnosing malaria. With the growing interest in developing in-field diagnostic devices that could be used by non-technical personnel involved, this study would surely provide the necessary preliminary data for innovating a device that can aid in the prevalence of malaria in the world. Because of that, a real-time PCR-based microfluidics platform that integrates and miniaturizes DNA purification, amplification, and detection is being introduced for in-field detection. This study aims to design and simulate a microfluidic PCR chip device that can specifically detect *Plasmodium falciparum* DNA fragment amplification using the software ANSYS software Computational Fluid Dynamics (CFD). The design is based on the real-time PCR amplification setting for conventional genus-specific PCR targeted on the 18S rRNA gene to detect malaria parasites (*Plasmodium falciparum*). The fluid property used in the simulation is based on the solvent property of water (see S3). Different materials, polypropylene, polycarbonate, and polydimethylsiloxane, were evaluated, and certain design considerations, such as the effects of looping and increasing the cross-sectional area, were analyzed. After simulating various models and conducting the necessary tests, this intends to determine from the results the most appropriate design that will help in pre-selecting the materials and planning the optimum parameters needed to consider before fabricating the microfluidic PCR chip device for actual use.

A similar goal was aimed by Zhao et al.; that is, they identified, assessed, and evaluated various microfluidic-based approaches in detecting foodborne pathogens. The designs were also intended to be miniaturized, portable, and low-cost. Some of the areas that were tackled in the study are the possible polymeric materials to use for the device, the sample

preparation in microfluidics, the application of the device to different technologies such as biosensors and PCRs, and the opportunities and challenges along with this innovation [16]. Another study by Wang et al. dealt with microfluidics-based strategies for diagnosing infectious diseases. It was also backed up by the same reason brought by the limitations of traditional methods in diagnoses. LOCC, LOAD, μPADs, and LFA microfluidic platforms were evaluated, and the application of digital nucleic acid assay for molecular diagnostics was highlighted [17].

Furthermore, a design and proof-of-concept study about magnetophoretic manipulation and separation of magnetic and non-magnetic particles in a ferro-microfluidic device was done, which results support the possibility of using magnetic excitation microfluidic system designs in analyzing cell separation [18]. To further realize the results obtained from the previous study, Hewlin et al. analyzed red blood cells and *E. coli* potential cell separation and sorting using the travelling wave ferro-magnetic microfluidic device. The dynamics of magnetic and non-magnetic entities with material magnetic susceptibility in a transient magnetic field was the focus of phase 2 of the investigation. From the results, the authors confirmed the potential efficiency of using the device in microparticles and cellular manipulation and sorting [19]. Through the utilization of CFD analysis and experimental cell culture growth based on the Huh7 cell line, the flow behavior and filling properties of two microfluidic liver-on-a-chip devices were examined and compared in another study by Bakuova et al. The two chips were subjected to computer evaluations, which revealed that the elliptical chamber chip that has been proposed in the work has better flow and filling characteristics than the circular chamber chip that has been previously described [20]. Some of these studies are evidence of the potential use of microfluidics in medical diagnoses and disease recognition. However, none has dwelled explicitly on the use of microfluidics PCR chips in the detection of malaria, which is the primary goal of this study.

Malaria is commonly diagnosed by microscopic examination using Giemsa-stained TBS, which is known as the gold standard method for malaria diagnosis, but this technique is not the best choice for low-level parasitemia and mixed infections [21,22]. However, in some endemic areas, asymptomatic infections are not usually detectable by microscopic examination. This limitation impacts malaria control and screening of blood samples [23]. Therefore, there is a need for a rapid and accurate diagnosis for effective treatment and control of malaria. It is necessary to develop diagnostic techniques with high sensitivity and specificity for detecting malaria in environments with relatively low parasite rates and among asymptomatic individuals of such disease [24]. Polymerase chain reaction (PCR) assay is found to be one of the most sensitive and specific methods in the detection of malaria parasites. A study was conducted to optimize a faster and cheaper real-time genus-specific PCR based on the 18S rRNA gene to detect malaria parasites at low grade parasitemia leading to a threshold sensitivity of 0.2 parasites per 1 μL [25]. However, the time interval concerning the collection and transportation of samples and the processing and dissemination of results limit the usefulness of PCR in routine clinical practice. Besides that, in most areas with malaria transmission in the Philippines, factors such as limited financial resources, persistent subclinical parasitemia, and inadequate laboratory infrastructures in the poor, remote rural areas impede PCR as a diagnostic method [26,27]. These factors are the reason for the need to create a microfluidic device that is portable, economical, and accessible but still functional like the conventional PCR is. With that, this study focuses on designing a microfluidic device that will mimic the function of a conventional genus-specific PCR based on the 18S rRNA gene to detect malaria parasites (*Plasmodium falciparum*) at low grade parasitemia.

2. Materials and Methods

The design was based on the DNA amplification procedure for conventional genus-specific PCR, whose target is the 18S rRNA gene in detecting malaria parasites (*Plasmodium falciparum*) at low grade parasitemia. The design requires 25 μL of PCR mix and 5 μL of DNA sample. The total volume of 30 μL then proceeds the annealing, extension, and

denaturation processes specified at various temperatures to determine which conditions the device would be most efficient to use (see S1 and S2).

Moreover, three polymers were tested to determine which material best suited the design for fabricating the microfluidic PCR chip. Table 1 lists the thermal properties of the polymeric materials utilized in the design.

Table 1. The types of polymeric material with their corresponding thermal properties are used in designing the microfluidic PCR chip.

Material Property	Unit	PP [a] [28]	PC [b] [29]	PDMS [c] [30]
Melting Point	K	432.15	428.15	408.15
Thermal Conductivity	$\frac{W}{m \cdot K}$	0.8	0.24	0.15
Specific Heat	$\frac{kJ}{kg \cdot K}$	1.8	1.2	1.46
Density	$\frac{kg}{m^3}$	920	1200	970

[a] *Polypropylene;* [b] *Polycarbonate;* [c] *Polydimethysiloxane.*

The first part of the simulation was intended to determine the effects of looping on the temperature of the fluid inside the device. Aside from that, this also aimed to assess the influence of looping on the efficiency of the design by calculating their respective relative deviation and average square of difference based on a set temperature of 58 °C. Three variations were tested: a design with no loops, one loop, and two loops of the same linear path lengths were drawn. The dimensions of these are shown in Table 2.

Table 2. The dimensions of the three designs utilized in the simulation.

	Total Length Calculation			
Design	**Radius (mm)**	**Circumference (mm)**	**Length (mm)**	**Total Length (mm)**
No Loops [a]	-	-	30	30
One Loop	0.25	1.570796327	14	30
Two Loops	0.25	1.570796327	27	30

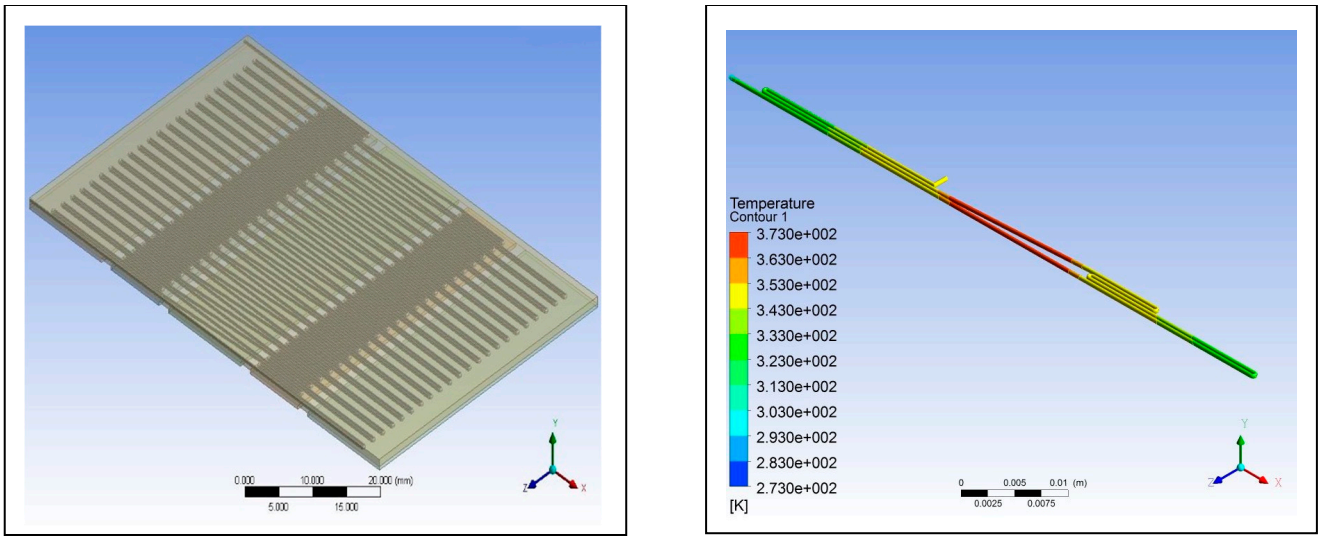
[a] *The design is characterized as having only a straight path line.*

The proposed microfluidic PCR chip was modeled using ANSYS 14.5 CFD with the following dimensions: the microfluidic chip with a length of 73 mm and width of 45.5 mm, denaturation with a length of 15 mm and width of 45.5 mm, annealing with a length of 15 mm and width of 45.5 mm, extension with a length of 10 mm and width of 45.5 mm, and the spaces with a length of 2 mm. The number of elements used was 512,000 because of license limitations. The mesh quality was kept greater than 0.10 to ensure quality for model simulations. The boundary conditions were adapted from Yang et al., wherein the Falkenhagen theory was used to evaluate the viscosity of the PCR mixture (see Supplementary) [31].

The other parameters considered in designing the microfluidic PCR chip are listed in Table 3. Moreover, Figure 1 shows the 44-cycle microfluidic PCR chip and the 2-cycle microfluidic PCR chip utilized in testing the design.

Table 3. Other parameters considered in the design of the microfluidic PCR chip.

Region	Residence Time (s)	Length (μm)	Passes	Volume	Volumetric Flowrate	Speed (μm/s)
Annealing (58 °C)	15	15,000	1.5	2.94×10^9	1.96×10^8	1500
Extension (72 °C)	20	10,000	3	3.92×10^9	1.96×10^8	1500
Denaturation (95 °C)	10	15,000	1	1.96×10^9	1.96×10^8	1500
Spaces	5.3	8000		1.05×10^9	1.96×10^8	1500
Total	153	75,500	3	9.87×10^9		



(**a**) (**b**)

Figure 1. Using ANSYS Computational Fluid Dynamics (CFD), the microfluidic PCR chip was modeled to evaluate which design would be best utilized in fabricating the device for actual use. The number of cycles must be specified since this also alters the functionality of the microfluidic PCR chip in detecting malaria. A visual comparison is shown: (**a**) 44-cycle microfluidic PCR chip design; (**b**) 2-cycle microfluidic PCR chip design.

Different polymeric materials, such as polypropylene, polycarbonate, and polydimethylsiloxane were used as the microfluidic PCR chip material in the simulation, and the most efficient material was evaluated using relative deviation and average square of difference. After assessing which material was the best choice, the diameter and length of the design were changed to determine the effect of varying the cross-sectional area of the fluid path to the fluid temperature. The first design has a diameter of 300 μm, a length of 200 μm, and a cross-sectional area of 130,686 μm^2. Meanwhile, the second design has a diameter of 500 μm, length of 300 μm, and cross-sectional area of 346,350 μm^2.

3. Results and Discussion

Designing and analyzing microfluidic PCR chips require a wide range of capabilities, which ANSYS provides. This enables engineers to evaluate various design factors and maximize chip performance by simulating complex fluid movement, heat transfer, and chemical reactions inside the device [32]. The software is appropriate for modelling biological reactions and fluid dynamics in microscale environments since it has advanced capacities like multiphase flow modeling, species transport, and surface reactions [33]. By using ANSYS, engineers may look at and improve on important elements, including channel shape, valve actuation, mixing effectiveness, and temperature management. They may check potential design problems and enhance chip performance before production

owing to the software's precise information on fluid velocity profiles, pressure distributions, residence duration, and temperature gradients [34,35]. Issues occurring while operating a chip can be found and mitigated using CFD. The potential for air bubbles, sedimentation, uneven heating, and uneven flow distribution, which can all have a detrimental impact on PCR performance and yield incorrect data, is part of this. The improved chip designs produced using CFD simulations result in higher sensitivity, decreased reaction time, and improved amplification efficiency, eventually improving the accuracy and reliability of molecular biology investigations on microfluidic PCR chips [36].

Using the data in Table 3, three designs were modeled, with one having no loops, one having only a loop, and one having two loops as seen in Figure 2. The copper plate for all three was set at a temperature of 58 °C, and the designs were simulated using CFD. As shown in Table 4, as the number of loops increased, the relative deviation also increased, which indicates that the efficiency of the design decreased; however, the decrease is not that significant. The simulation shows that a fluid temperature maintained within 58 °C, the relative deviation for the design with no loops is 0.65, with one loop is 0.80, and with two loops is 0.88. This decreasing trend is indicative of decreasing efficiency but only to a minimal extent. Therefore, looping can be used in designing the microfluidic PCR chip to reduce the chip length and increase the residence time of the sample inside the device.

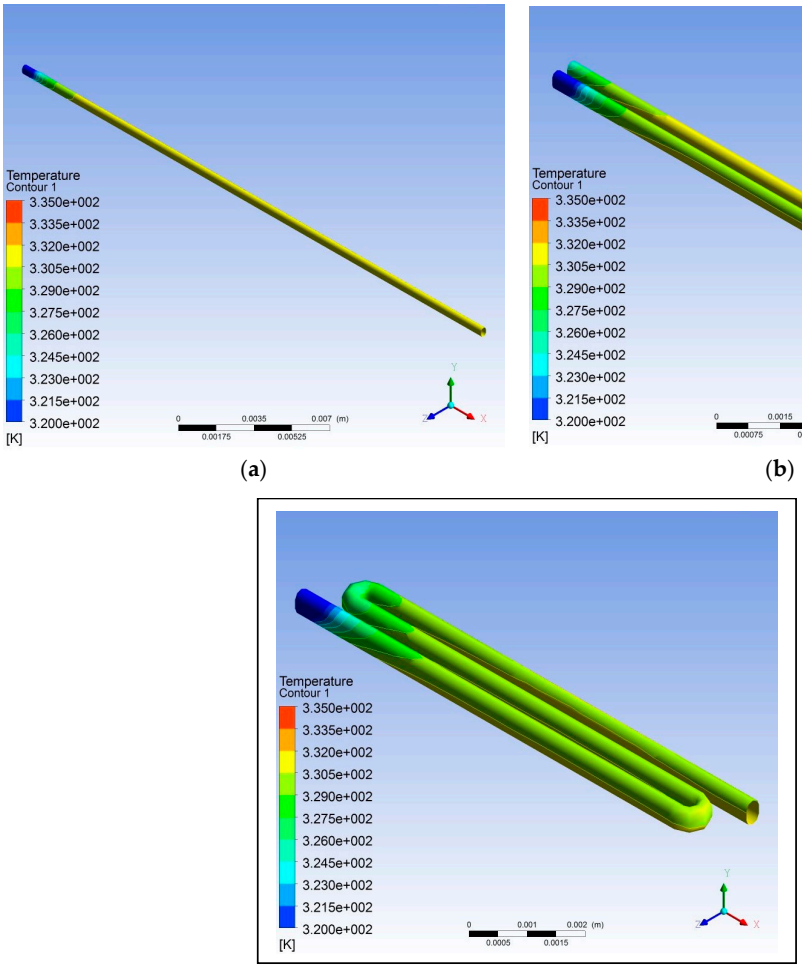


Figure 2. Using ANSYS Computational Fluid Dynamics (CFD), the designs intended to test the effect of looping on the efficiency of the device were modelled: (**a**) Design with no loops; (**b**) Design with one loop; (**c**) Design with two loops.

Table 4. The effect of looping on the efficiency of the design is based on the calculation of relative deviation and ASD.

Design	Relative Deviation	ASD [a]
No Loop	0.65	1.12
One Loop	0.80	1.39
Two Loops	0.88	1.52

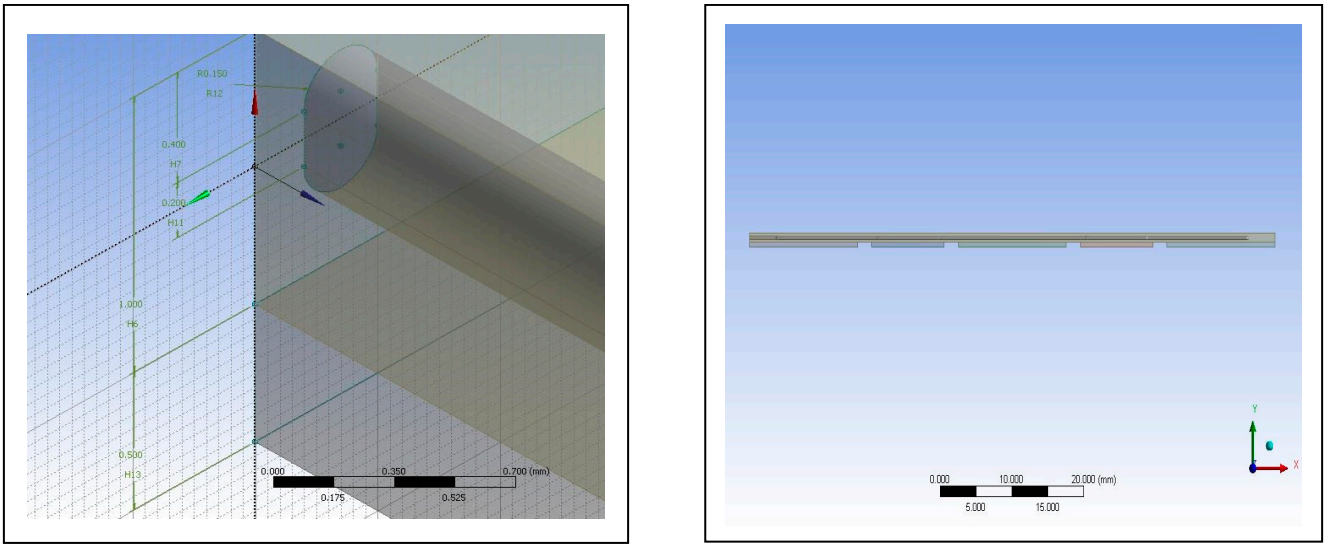
[a] *Average square of difference* $(\Delta T^2/n)$.

The efficiency of reactant mixing is improved by looping in the design of microfluidic PCR chips, which boosts amplification performance [7,37]. Looping enables the controlled transport and dispersion of target DNA, primers, and enzymes by providing circular flow channels within the chip. The looping structures induce vortices and turbulence that speed up the reaction rate by promoting reactant collision and contact [38]. Additionally, looping lessens the effects of diffusion and permits the complete mixing of the reactants, even in small volumes. This enhances the reaction's homogeneity and lessens the possibility of non-specific amplification. To improve mixing efficiency within the microfluidic PCR chip, looping design solutions, such as serpentine channels or spiral topologies, efficiently harness the advantages of fluidic looping [39,40]. The regulation of temperature cycling, a crucial component of PCR, can be enhanced by adding more loops. The flow of chemicals can be guided by the loops through several temperature ranges, enabling quick and precise changes between denaturation, annealing, and extension temperatures. By doing so, thermal lag is avoided, and temperature fluctuations in the reaction mixture are reduced, essential for maintaining the integrity of DNA strands and achieving the best possible primer annealing. The loops make it possible for PCR amplification to be more reliable and effective by efficiently managing heat transmission [41,42]. Also, a better level of reaction consistency may result from a certain number of loops. Even slight changes in reagent concentrations, temperature profiles, or mixing effectiveness during microfluidic PCR can have a significant impact on the results of the reaction. The microfluidic system may self-correct and achieve redundancy with more loops. Other loops can make up for a minor interruption or fluctuation in one loop, ensuring that the total reaction doesn't get off course. This self-stabilizing characteristic improves the PCR process's reproducibility and dependability, which is significant in research and diagnostic applications where precision is essential [42].

For the simulation of the proposed microfluidic PCR chip, the designs drawn using ANSYS are shown in Figure 3. The five copper plates seen in Figure 3b were set at temperatures 58 °C, 72 °C, 95 °C, 72 °C, and 58 °C for the first simulation, temperatures 60 °C, 74 °C, 97 °C, 74 °C, and 60 °C for the second simulation, and temperatures 63 °C, 77 °C, 100 °C, 77 °C, and 63 °C for the third simulation for each material (the order of temperatures corresponds to the order of plates seen in the figure).

Thermal cycling involves heating and cooling the reaction mixture at particular temperatures for DNA amplification, an essential procedure in microfluidic PCR chips. Thermal cycle efficiency can be significantly improved by using looping design concepts. The chip design can enhance the surface area accessible for heat transfer by including loops and complicated routes [43]. As a result, the fluid and the chip material surrounding it may exchange heat more effectively, improving the temperature homogeneity of the reaction mixture [44]. For reliable and constant amplification results, efficient heat dissipation and minimal temperature changes are made possible by the looping structures. The vast range of simulation tools provided by ANSYS CFD is essential for creating microfluidic PCR chips with the best possible thermal cycling [45]. The software enables engineers to evaluate and optimize numerous design parameters by simulating heat transfer, fluid flow, and temperature distribution within the chip. Engineers can examine the effects of chip geometry, channel dimensions, and materials on temperature distribution during thermal cycling with simulations [46]. The software helps identify potential hotspots or temperature changes that could impact the PCR process by providing insights into variables, including

convective heat transfer, conductive heat dissipation, and radiative heat exchange [47]. By offering a virtual environment to investigate and improve different thermal cycle factors, CFD considerably cuts down on the time and expense of experimental trial and error [48]. Because it can mimic various temperature profiles, cycling rates, and thermal profiles, chip designs may be evaluated and optimized quickly.



(**a**) (**b**)

Figure 3. Images of the proposed microfluidic PCR chip design modelled from ANSYS Computational Fluid Dynamics (CFD): (**a**) Microfluidic chip dimensions; (**b**) Microfluidic chip side view.

Additionally, it provides in-depth representations of temperature distributions, enabling users to see potential temperature changes or places with inadequate temperature control [49]. To maintain uniform temperature distribution and reduce temperature swings during thermal cycling, designers can optimize chip shape, channel layout, and thermal management strategies by understanding the heat transfer processes within the chip [50]. The evaluation of chip performance under various operating conditions is made easier with CFD. To assess the durability and reliability of the microfluidic PCR chip design, it can simulate changes in sample volume, flow rates, and ambient temperature [51]. The detection of possible problems and the tuning of thermal cycle parameters for improved performance are made easier with this information.

The final design is illustrated in Figure 4, showing a 44-cycle microfluidic PCR chip design.

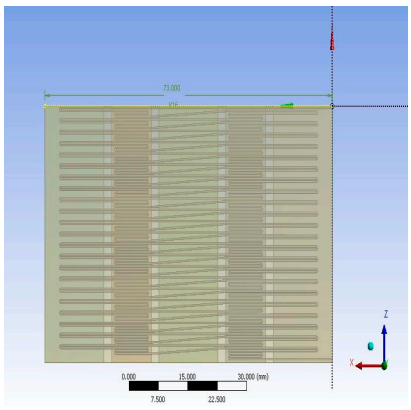


Figure 4. Image of the 44-cycle microfluidic PCR chip design modelled from ANSYS Computational Fluid Dynamics (CFD).

In microfluidic PCR chips, the cycle number is crucial for maximizing amplification effectiveness. The possibility of DNA amplification is increased by increasing the number of cycles, especially for low-copy targets [52]. The concentration of the target DNA molecules rises exponentially as the number of cycles is increased, enhancing sensitivity and detection limits [53]. However, to prevent non-specific amplification, which might produce false-positive results, the cycle number must be properly noted. When unwanted DNA fragments are amplified with the target DNA, this is known as non-specific amplification [54]. By making sure that the target sequence is specifically amplified, optimizing the cycle number improves the accuracy of the PCR chip.

Furthermore, the entire reaction time needed for DNA amplification in microfluidic PCR chips is directly influenced by the number of cycles [55]. The overall reaction time increases as the cycle number rises, which might be an important factor for applications that require lower reaction time. By maximizing thermal cycling efficiency, decreasing heat transfer delays, and increasing temperature uniformity, microfluidic PCR chip designs should seek to speed up reaction times [56]. Using the computational fluid dynamics program ANSYS CFD, thermal cycling in microfluidic PCR chips may be simulated and optimized, allowing the right cycle number to produce the required amplification in the least time [57]. The power usage, as well as the heating and cooling requirements of the PCR chip, are influenced by the cycle number. Higher cycle numbers could necessitate more energy and stricter temperature control, impacting chip design factors, including power supply, thermal management, and overall system complexity. It is crucial to carefully consider the desired DNA concentration, amplification sensitivity, time restrictions, and other performance requirements when determining the ideal cycle number for a given application [58,59]. A balance between amplification efficiency, specificity, reaction time, and chip performance can be achieved by taking these parameters into account.

However, during the meshing procedure on the ANSYS, the software cannot mesh the design due to its limitation since the program is only accessible for educational use. Instead of meshing the whole design, only a 2-cycle microfluidic PCR chip design was meshed and simulated as representative of the 44-cycle microfluidic PCR chip design.

Furthermore, different polymeric materials were tested to determine which is the best choice to be used for designing the microfluidic chip. Polypropylene demonstrates several significant benefits that make it suitable for microfluidic PCR chip design. Polypropylene is chemically inert, making it extremely resistant to various chemicals and solvents used in PCR operations [60]. This characteristic guarantees that polypropylene does not affect the amplification reaction, resulting in precise and trustworthy results. Polypropylene is also biocompatible, which reduces the chance of sample contamination and offers an ideal setting for PCR reactions. Comparing polypropylene to other materials like glass or silicon, it is more affordable. Microfluidic PCR chips may be produced in large quantities owing to their affordable manufacturing costs, increasing their availability to researchers and healthcare workers [61].

Additionally, polypropylene is simple to shape, making it possible to create intricate patterns with exact geometries and microfluidic elements. Furthermore, a crucial factor for PCR applications is heat stability. Repeated heating and cooling cycles are necessary for microfluidic PCR chips to achieve DNA denaturation, annealing, and extension. With a melting point of roughly 160–170 °C and a glass transition temperature of roughly −10 °C, polypropylene has good thermal stability [62]. With the aid of these characteristics, the microfluidic PCR chip can resist the temperature cycling necessary for PCR without deforming or losing structural integrity. Due to the poor thermal conductivity of polypropylene, there is less heat transmission between the various areas of the microfluidic chip, which allows for effective temperature control and less sample contamination [63]. By maintaining well-defined heat zones for each reaction step, this characteristic guarantees that the PCR process is accurate and specific.

Real-time observation and analysis of PCR reactions are made possible by the optical transparency of polycarbonate. This characteristic allows researchers to monitor and mea-

sure the amplification procedure, leading to precise and accurate results. Polycarbonate has exceptional chemical resistance, making it work with various PCR reagents and lowering the possibility of sample contamination [64]. The microfluidic PCR chip is long-lasting due to its resistance to chemical deterioration, making it a good material for recurrent use. Also, polycarbonate is non-toxic and biocompatible, limiting disruption of biological material and maintaining the integrity of DNA amplification [65]. These qualities also make polycarbonate a suitable material for microfluidic PCR chip applications since they are essential for preserving the precision and dependability of PCR results. Because polycarbonate has a low thermal conductivity, there is less heat transfer between the various parts of the microfluidic chip. This characteristic makes it easier to effectively control the temperature within designated reaction zones, reducing the risk of cross-contamination and preserving the precision and specificity of the PCR procedure [66]. The temperature cycling of the microfluidic PCR chip is more consistent due to the low coefficient of thermal expansion of polycarbonate. Enhancing the reliability of PCR results and minimizing fluctuations in DNA amplification efficiency are temperature profiles that are constant across the chip. The microfluidic PCR chip design can also benefit from the hydrophobic properties of the polymer [67]. It does not require further surface alterations or coatings because of its inherent hydrophobicity, which enables precise control of fluid flow. This characteristic lowers the possibility of sample evaporation during the PCR process and improves the compatibility of polycarbonate microfluidic PCR chips with PCR chemicals [68].

Due to its great flexibility and elastomeric properties, polydimethylsiloxane (PDMS) makes it simple to fabricate intricate microfluidic devices. It is simple to mold or pattern to make complex features and structures, which makes it easier to incorporate several functionalities into a single chip [64,69]. The adaptability and use of microfluidic PCR chips are increased by the flexibility of PDMS, which also makes it possible to connect tubing and other components in a simple manner. In comparison to other materials like glass or silicon, PDMS is also a more affordable choice. Microfluidic PCR chips can be produced in large quantities thanks to their affordable manufacturing costs, which increases their accessibility for researchers and medical experts [70]. Precision temperature control within designated reaction zones is made possible by the poor thermal conductivity of PDMS, which reduces heat transmission between various areas of the microfluidic chip. This characteristic improves the precision and specificity of the PCR process and lowers the possibility of sample contamination. Due to its low coefficient of thermal expansion, PDMS also provides good thermal cycling performance [71]. With this feature, the microfluidic PCR chip is guaranteed to have constant temperature profiles, which minimizes changes in DNA amplification efficiency and enhances the repeatability of results.

Additionally, PDMS demonstrates hydrophobic characteristics that can be used to regulate fluid flow inside the microfluidic device [72]. The hydrophobic properties of PDMS make it easier for well-defined channels and droplets to develop, providing fine-grained control over the transport of samples and reagents [73]. This characteristic lowers the possibility of sample evaporation during the PCR process and improves the compatibility of PDMS microfluidic PCR chips with various PCR reagents.

Using the data at different nodes generated from the various simulations, the relative deviation and average square of difference (ASD) were calculated as shown in Table 5. The results show that polypropylene set at a copper plate temperature of 58 °C for annealing, 72 °C for extension, and 95 °C for denaturation has the lowest relative deviation of 0.94 and lowest average square of difference ($\Delta T^2/n$) of 3.21. This suggests that polypropylene under this set condition, is the most efficient material for fabricating the microfluidic PCR chip.

Table 5. The effect of the different polymeric materials under various temperatures on the efficiency of the design is based on the calculation of relative deviation and ASD.

	58 °C, 72 °C, 95 °C		60 °C, 74 °C, 97 °C		63 °C, 77 °C, 100 °C	
Material	**Relative Deviation**	**ASD** [d]	**Relative Deviation**	**ASD** [d]	**Relative Deviation**	**ASD** [d]
PP [a]	0.94	3.21	2.84	5.82	6.90	25.00
PC [b]	1.92	8.61	3.15	10.00	6.92	26.70
PDMS [c]	2.78	14.00	3.47	14.00	6.93	29.00

[a] *Polypropylene;* [b] *Polycarbonate;* [c] *Polydimethysiloxane;* [d] *Average square of difference* $(\Delta T^2/n)$.

The relative deviation was intended to measure the precision or the consistency of results that can be generated from the designed microfluidic PCR chip. It measures the variance between subsequent readings of the same measurement or replication of the DNA amplification procedure [74]. An extremely high degree of precision and reproducibility is implied by a low relative deviation, which shows little variation in the amplification results. Achieving a minimal relative variation in the design of microfluidic PCR chips is essential for precise and dependable DNA amplification. It indicates that there is less chance of false-positive or false-negative results because of the ability of the PCR chip to enable consistent and repeatable results [75]. In applications like diagnostics or research trials, where consistency and reproducibility are crucial, a low relative deviation assures that the chip generates accurate data.

Conversely, the average square of difference was intended to measure the accuracy or efficiency of the proposed design. It measures the difference between the actual outcomes of the amplification and the desired or anticipated values. The chip design produces accurate amplification results with little departure from the expected values, as indicated by a low average square of difference. To achieve precise DNA amplification while designing microfluidic PCR chips, a low average square of difference must be attained [76]. A low value denotes effective reaction parameter control by the chip design, including control of temperature, reagent concentrations, and fluid flow, leading to accurate and dependable amplification [77]. The likelihood of experimental mistakes, erroneous results, or the necessity for additional testing is reduced by a precise chip design.

Consequently, after figuring out that polypropylene is the best option, the microfluidic PCR chip with this design set under the optimum temperatures of 58 °C, 72 °C, and 95 °C was subjected to further testing by changing the parameters while unchanging the inlet velocity of the models. Table 6 shows the relative deviation and average square of difference (ASD) calculated from the two designs.

Table 6. The effect of changing the parameters of the polypropylene microfluidic PCR chip under the temperatures of 58 °C (annealing), 72 °C (extension), and 95 °C (denaturation) on the efficiency of the design based on the calculation of relative deviation and ASD.

Parameter	**Design 1**	**Design 2**
Diameter	300	500
Length	200	300
Relative Deviation	0.94	2.31
ASD [a]	3.21	5.96

[a] *Average square of difference* $(\Delta T^2/n)$.

The data shows that increasing the diameter and length of the fluid path affects the fluid temperature. Increasing the cross-sectional area using the same inlet velocity of the fluid flowing through the chip also increases relative deviation and ASD, indicating a decrease in efficiency of reaching the set temperature at different zones. Thus, the design using a smaller cross-sectional area is most suitable because of its comparatively lower relative deviation, and ASD predisposes it to be favored more in fabricating the device.

Since one of the aims of this study is to reduce the cost of the device for public access, limiting material utilization was necessary to compensate for this. Miniaturizing the conventional PCR by fabricating a more portable and relatively smaller device would surely affect its efficiency in detecting Malaria, especially in the amplification process [78]. The proposed microfluidic PCR chip is designed to detect malaria even with a small sample volume while, at the same time, not compromising the specificity of the test. The threshold cycle of the conventional PCR amplification of *P. falciparum* DNA fragments is 36, with a threshold sensitivity of 0.2 parasites per 1 µL [79]. However, the proposed microfluidic PCR chip was designed for 44 cycles because it is also intended to detect small samples, which must be amplified more than samples of greater volume. The amount of time it takes for the microfluidic PCR chip to amplify the sample for a given number of cycles was evaluated (see S3). In 44 cycles, which the device was specifically designed for, it consumes around 112.23 min to complete.

4. Conclusions

The simulation shows that looping can affect the temperature of the fluid to a minimal extent and, therefore, can be considered in designing a microfluidic PCR chip to decrease the chip length. Based on the simulation, the best material for designing the microfluidic PCR chip is polypropylene with a relative deviation of 0.94 from the set temperatures of 58 °C (annealing), 72 °C (extension), and 95 °C (denaturation). It was also proven that increasing the cross-sectional area of the fluid path can affect the temperature of the fluid; thus, it is recommended to use a smaller cross-sectional area to ensure that the set temperatures in different zones are reached. With the objectives this simulative study aims to answer, the results generated from this intend to serve as a preliminary screening towards an optimized design of a microfluidic-based PCR device for *P. falciparum* DNA fragment amplification.

As mentioned earlier, only a 2-cycle microfluidic chip design was meshed due to the limitation of the software. Future researchers might want to look at this and try to mesh the 44-cycle microfluidic chip used in this study. Other parameters may be tested as well for their effect on the efficiency of the device in detecting malaria, and other materials may be assessed to broaden the application of this design.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/computation11100190/s1, S1: Optimized Real-Time PCR Procedure for Malaria Detection in Low Level Parasite; S2: Viscosity Calculation; S3: Design Calculation.

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