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Development of a Self-Regulating Paper-Based Microfluidic Platform for Instrument-Free Molecular Diagnostics

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Abstract

This thesis addresses the engineering gap between laboratory-based nucleic-acid amplification tests and low-cost lateral-flow diagnostics by developing an instrument-free paper-based platform for amplified respiratory-virus detection. The work investigated three linked technological questions: whether a printed positive temperature coefficient ink heater could passively maintain the target thermal window without active feedback electronics; whether a dot-array nucleic-acid lateral-flow architecture could reduce the spatial and reagent-depletion limitations of conventional multi-line strips; and whether a thermally actuated wax gate could create a passive time delay to synchronise amplification with downstream detection.

A stencil-printed paper heater combining silver interdigitated electrodes with carbon-based positive temperature coefficient ink was developed and tuned for low-voltage operation. The heater exploited the intrinsic resistance increase of the composite to provide passive thermal regulation within the compatible range. A five-target dot-array lateral-flow strip was then developed for SARS-CoV-2, Influenza A/B and RSV A/B amplicons. Compared with the conventional line format, the dot-array layout reduced downstream signal depletion and enabled compact multiplex readout. On-paper amplification studies identified cellulose filter paper as a more suitable amplification and amplicon-release matrix than glass fibre. Finally, a thermally coupled wax-gate device was developed in which gate width programmed the actuation delay, allowing amplification products to be released only after the incubation period.

The integrated single-target prototype detected Influenza B synthetic DNA at an observed limit of detection of 200 copies total input. The work is presented as proof-of-principle engineering using synthetic DNA templates only; no recovery, extraction or purification of nucleic acids from clinical samples was performed. The thesis contributes design rules for printed self-regulating heating, dot-array nucleic-acid lateral-flow multiplexing, and passive thermal-fluidic synchronisation for future sample-to-answer paper diagnostics.

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This one is for all of you.

Declaration

I certify that the thesis presented here for examination for a PhD degree of the University of Glasgow is solely my own work other than where I have clearly indicated that it is the work of others (in which case the extent of any work carried out jointly by me and any other person is clearly identified in it) and that the thesis has not been edited by a third party beyond what is permitted by the University's PGR Code of Practice.

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I acknowledge that if any issues are raised regarding good research practice based on review of the thesis, the examination may be postponed pending the outcome of any investigation of the issues.

Date: 10th March 2026

Signature: Jiaxing Huang

Abbreviations

- AF488 - Alexa Fluor 488
- ASSURED - Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable
- AuNP - Gold Nanoparticle
- B3 - Backward Outer Primer
- BIO - Biotin
- BIP - Backward Inner Primer
- bp - Base Pairs
- CEO - Centre D'Estudis D'Opinion, Catalan Centre of Opinion Studies
- CFU - Colony-Forming Unit
- COVID-19 - Coronavirus Disease 2019
- CRISPR - Clustered Regularly Interspaced Short Palindromic Repeats
- DC - Direct Current
- DI - Deionised
- DIG - Digoxigenin
- DNA - Deoxyribonucleic Acid
- DNP - Dinitrophenyl
- dNTP - Deoxynucleoside Triphosphate
- dsDNA - Double-Stranded DNA
- EMI - Electromagnetic Interference
- F3 - Forward Outer Primer
- FAM - 6-Carboxyfluorescein
- FIP - Forward Inner Primer
- FITC - Fluorescein Isothiocyanate
- GF - Glass Fibre / Glass Microfiber
- HDA - Helicase-Dependent Amplification
- IDE - Interdigitated Electrode
- IgG - Immunoglobulin G

-
- Inf B - Influenza B
 - LAMP - Loop-Mediated Isothermal Amplification
 - LB - Backward Loop Primer
 - LED - Light-Emitting Diode
 - LF - Forward Loop Primer
 - LFA - Lateral Flow Assay
 - LFS - Lateral Flow Strip
 - LOD - Limit of Detection
 - MIH - Magnetic Induction Heating
 - μ PAD - Microfluidic Paper-based Analytical Device
 - NA - Nucleic Acid
 - NAAT - Nucleic Acid Amplification Test
 - NALFS - Nucleic Acid Lateral Flow Strip
 - NC - Nitrocellulose
 - NTC - No Template Control
 - OD - Optical Density
 - PBS - Phosphate-Buffered Saline
 - PCB - Printed Circuit Board
 - PCM - Phase-Change Material
 - PCR - Polymerase Chain Reaction
 - PDMS - Polydimethylsiloxane
 - PES - Polyethersulfone
 - PID - Proportional-Integral-Derivative
 - PLA - Polylactic Acid
 - PMMA - Polymethyl Methacrylate
 - POC - Point-of-Care
 - PTC - Positive Temperature Coefficient
 - qPCR - Quantitative Polymerase Chain Reaction
 - R2R - Roll-to-Roll
 - REASSURED - Real-time connectivity, Ease of specimen collection, Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable
 - RNA - Ribonucleic Acid

- RPA - Recombinase Polymerase Amplification
- RSV - Respiratory Syncytial Virus
- RT-LAMP - Reverse Transcription Loop-Mediated Isothermal Amplification
- SA - Streptavidin
- SA-Au - Streptavidin-Gold
- SA-AuNP - Streptavidin-conjugated Gold Nanoparticle
- SARS-CoV-2 - Severe Acute Respiratory Syndrome Coronavirus 2
- ssDNA - Single-Stranded DNA
- TAMRA - Tetramethylrhodamine
- TE - Tris-EDTA
- tHDA - Thermophilic Helicase-Dependent Amplification
- USB - Universal Serial Bus
- UV - Ultraviolet
- UV-vis - Ultraviolet-Visible
- WHO - World Health Organization

Chapter 1

Introduction

1.1 Clinical Background and Motivation

1.1.1 The Burden of Respiratory Viruses and the Challenge of Co-circulation

Respiratory viral infections continue to represent a profound challenge to global public health and economic stability. Among the diverse array of pathogens, Influenza viruses (types A and B), Respiratory Syncytial Virus (RSV), and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) are the primary agents responsible for severe acute respiratory infections (ARIs) (Xie, Zong, *et al.* 2025). These pathogens disproportionately affect vulnerable groups, particularly older adults, in whom an aging immune system and the presence of chronic health conditions increase the risk of serious illness (Hanage *et al.* 2024).

In the post-COVID-19 era, the epidemiological landscape has shifted significantly. While public health measures initially lowered the presence of traditional respiratory viruses, recent monitoring indicates a resurgence and simultaneous circulation of Influenza, RSV, and SARS-CoV-2 (Contes *et al.* 2025). This co-circulation increases the risk of co-infections, which can increase a patient much sicker through immune system overload and viral interference. Such interactions can lead to more extensive lung tissue damage compared to infections caused by a single virus (Golpour *et al.* 2025).

Clinically, these infections present with overlapping, non-specific symptoms (such as fever, cough, fatigue, and shortness of breath), rendering them nearly indistinguishable based on a physical examination (Chakraborty 2024). This similarity in symptoms creates a major risk for patient care because treatments depend on knowing exactly which virus is present. For example,

antivirals like oseltamivir are effective for Influenza but do not work against RSV or SARS-CoV-2 (Contes *et al.* 2025). Consequently, rapid and accurate testing that can distinguish between these viruses is essential to ensure patients receive the correct medication and to stop the spread of infection (Hanage *et al.* 2024).

1.1.2 Limitations of Current Diagnostics: The Sensitivity vs. Accessibility Trade-off

The current diagnostic framework is split between high-quality laboratory testing and easy-to-access rapid tests. The “gold standard” for identifying respiratory viruses is the Reverse Transcription Polymerase Chain Reaction (RT-PCR). This method is highly sensitive and specific, meaning it can detect even very small amounts of the virus during different stages of the infection (K. P. Jiang *et al.* 2024). However, RT-PCR is limited because it requires expensive machines that cycle through different temperatures, highly trained staff, and a laboratory setting. This usually results in wait times for results ranging from several hours to several days (J. Tang *et al.* 2024). These delays make it difficult for doctors to make immediate treatment decisions in local clinics or for patients to use the technology at home.

To provide faster results, rapid antigen tests based on lateral flow technology have been widely used. While these tests are low-cost, simple to use, and provide results in 15–30 minutes, they often lack the accuracy of molecular tests (H.-J. Lim *et al.* 2023). Studies show that these rapid tests often give false-negative results in patients who have less of the virus in their system, which can allow the virus to continue spreading unnoticed (J. Tang *et al.* 2024). Therefore, a major technological gap exists for a test that is as easy to access as a rapid antigen test but as accurate as a PCR test.

1.1.3 The Goal of Instrument-Free Diagnostics: From ASSURED to REASSURED

To help create better point-of-care (POC) tests that solve these problems, the World Health Organisation (WHO) created the ASSURED criteria, which stands for Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free, and Deliverable. This list has recently been updated to the REASSURED criteria to include modern needs for digital technology

and easier sampling (Chakraborty 2024). The updated criteria specifically highlight the importance of real-time connectivity to transmit data for disease tracking and the use of easier sample collection methods, such as saliva or simple nasal swabs, to make self-testing more practical (Lamprou *et al.* 2025).

The ultimate goal of this engineering field is to develop “Instrument-Free” molecular tests. These platforms aim to perform high-quality DNA or RNA amplification inside a low-cost disposable device that operates without external electricity or bulky readers, thereby satisfying the REASSURED criteria while ensuring high diagnostic accuracy in resource-limited settings (Chakraborty 2024).

1.2 State of the Art

1.2.1 Nucleic Acid Lateral Flow Strip

Principle of Operation

The Nucleic Acid Lateral Flow Strip (NALFS) adapts the established architecture of immunochromatographic assays to detect specific genetic amplicons rather than proteins. Unlike rapid antigen tests, which rely on the direct binding of antibodies to viral surface proteins (e.g., Spike or Nucleocapsid proteins) and frequently suffer from limited sensitivity in samples with low viral loads, NALFS serves as the visual readout for Nucleic Acid Amplification Tests (NAATs) (K. Wang *et al.* 2024). This combination leverages the exponential amplification of target DNA or RNA to achieve high analytical sensitivity while maintaining the equipment-free simplicity of lateral flow devices (Chakraborty 2024). The schematic architecture of a conventional lateral flow strip is presented in Figure 1.1(A), while the comparative principles of rapid antigen tests and NALFS are illustrated in Figure 1.1(B).

The most prevalent detection mechanism in NALFS is the hapten-mediated “sandwich” assay. In this format, the amplification reaction utilises primers labelled with distinct haptens, typically Biotin and a specific antigen such as Fluorescein Isothiocyanate (FITC), Digoxigenin (Dig), or 6-Carboxyfluorescein (FAM) (Garg *et al.* 2022). As the reaction mixture wicks through the strip, the Biotin-labelled end of the dual-labelled amplicon binds to streptavidin-conjugated reporters (e.g., gold nanoparticles, AuNPs) released from the conjugate pad. This complex mi-

grates along the nitrocellulose membrane to the test line, where immobilised antibodies specific to the second hapten (e.g., anti-FITC or anti-Dig) capture the amplicon-reporter complex (Reynolds *et al.* 2023). The accumulation of AuNPs at the test line produces a characteristic red band, providing a binary “yes/no” result visible to the naked eye (Lamprou *et al.* 2025).

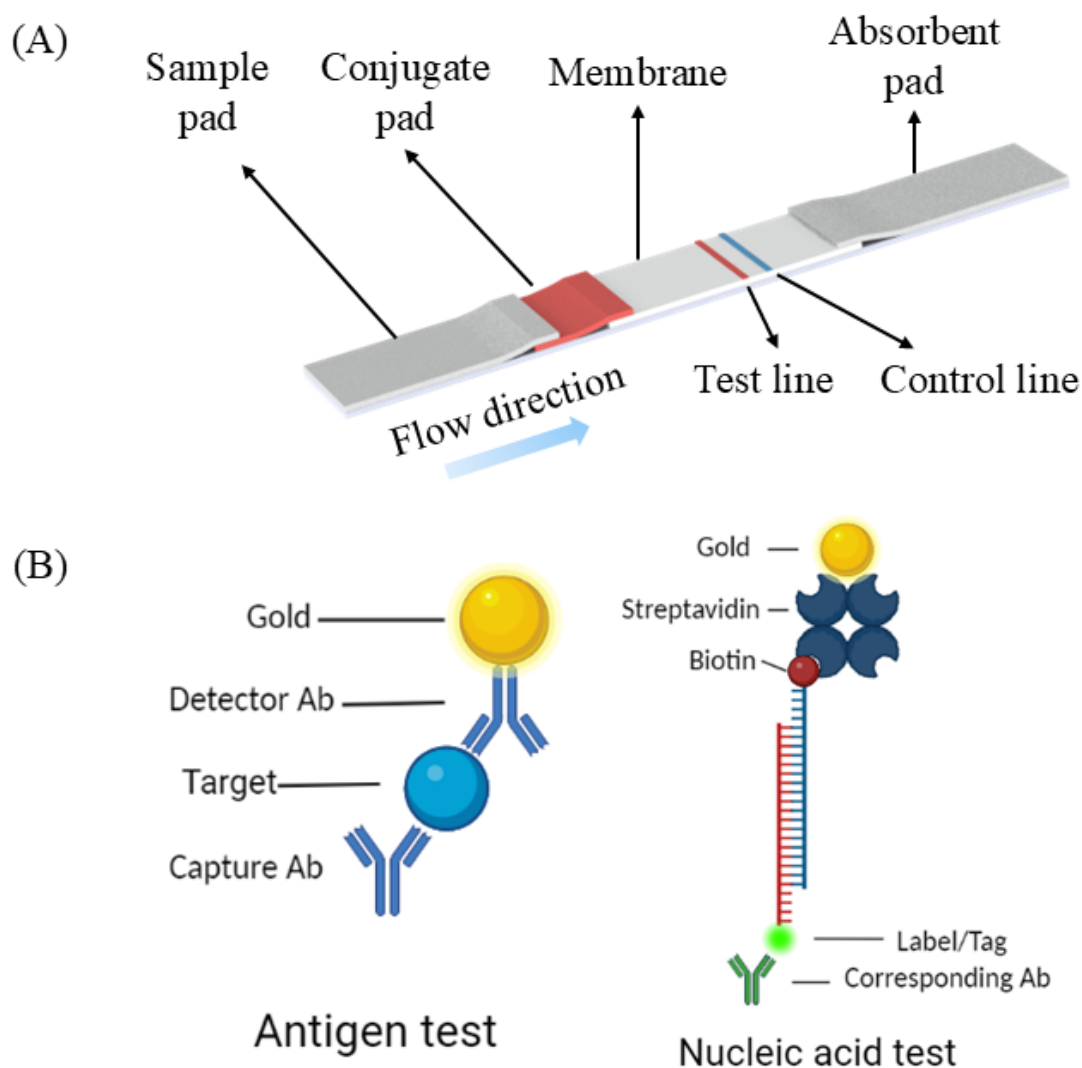


Figure 1.1: **Schematic illustration of lateral flow assay principles.** (A) Standard architecture of a lateral flow strip, showing the overlapping sequence of the sample pad, conjugate pad, nitrocellulose membrane hosting Test (T) and Control (C) lines, and the downstream absorbent pad. (B) Comparative detection principles. The left panel illustrates a protein-based Rapid Antigen Test detecting viral antigens directly using labelled antibodies. The right panel illustrates a Nucleic Acid Lateral Flow Strip (NALFS), which detects dual-labelled genetic amplicons (e.g., labelled with Biotin and FITC during upstream amplification) using a sandwich assay format involving streptavidin-conjugated reporters (e.g., SA-AuNPs) and immobilised anti-hapten capture antibodies.

Challenges in Multiplexing

While NALFS is robust for single-target detection, expanding this architecture to multiplexed detection (simultaneously identifying multiple pathogens or gene targets on a single strip) introduces complex fluidic and biochemical challenges. The conventional strategy of placing multiple test lines sequentially along the flow path is limited by membrane length and capillary flow dynamics. In a lateral flow strip (LFS), the flow rate decays as the liquid front advances. Consequently, adding more lines necessitates longer strips, which significantly increases assay time and can lead to flow instability, where closely spaced signals become indistinguishable (Hofmann *et al.* 2021). Furthermore, sequential capture zones create a “depletion effect”, in which high concentrations of an upstream target consume most of the available reporter conjugates (e.g., SA-AuNPs), leaving insufficient reagents for downstream targets and potentially leading to false negatives (Pedreira-Rincón *et al.* 2025). Conversely, extremely high amplicon concentrations can induce a “hook effect”, where free labelled amplicons saturate the capture antibodies without binding to the reporter nanoparticles, leading to paradoxically reduced signal intensity (Kakkar *et al.* 2024).

Strategies for Multiplexed LFS Design

To overcome these limitations, researchers have developed various architectural strategies to facilitate multiplexing in LFSs:

- **Spatial Multiplexing (Multi-Line):** This is the standard approach, arranging distinct capture antibodies in parallel lines perpendicular to the flow. While early literature suggested a practical limit of 2–3 targets due to spatial constraints and flow deceleration, recent optimisations in membrane selection and buffer rheology have expanded this capability. For instance, Lamprou *et al.* reported that optimised spatial arrangements enabled the detection of up to five distinct targets on a single strip (Lamprou *et al.* 2025).

Although extending this linear format beyond five targets (e.g., 8–10 test lines) has been demonstrated (M. Lin *et al.* 2025), these high-density configurations necessitate rigorous empirical optimisation. To counteract the reporter consumption, such assays require a disproportionately high conjugate load to ensure that sufficient reporter nanoparticles

reach the distal test lines. Furthermore, the capture antibody concentrations must be meticulously balanced to prevent the proximal lines from sequestering all available targets, a trade-off that often increases the overall cost and complexity of the device (Pedreira-Rincón *et al.* 2025).

- **Dot-Array and 2D Patterning:** To mitigate flow resistance and increase target density, researchers have shifted from lines to micro-spotted arrays or 2D patterns. This geometry reduces the flow resistance per capture zone and allows fluid to flow around saturated spots, thereby preserving reagent availability for downstream targets. A seminal example is the work by Taranova *et al.*, who successfully demonstrated the simultaneous detection of 32 different analytes on a single strip using a microarray-style dot pattern (N. A. Taranova *et al.* 2013). Similarly, Hofmann *et al.* developed a “Blind Spot” Braille-patterned LFA where reagents are printed as pixelated dots parallel to the flow direction. This geometry reduces flow retardation and allows for the simultaneous detection of multiple biothreat agents without the “shadowing” effects seen in sequential lines, proving that 2D spatial encoding can significantly increase information density without compromising assay kinetics (Hofmann *et al.* 2021).
- **Multi-Channel Architectures:** To avoid cross-reactivity and reagent competition entirely, strips can be designed with parallel channels (Y. Zhao *et al.* 2016). In these configurations, a single sample inlet distributes the fluid into physically separated channels, each dedicated to a specific target (Kakkar *et al.* 2024). While this eliminates reagent depletion issues, it typically increases the required sample volume and device footprint (Chakraborty 2024).
- **Spectral Multiplexing:** Beyond spatial separation, multiplexing can be achieved using reporters with distinct optical signatures, such as quantum dots (QDs, N. Taranova *et al.* 2015) or upconversion nanoparticles (UCNPs, Jin *et al.* 2018) that emit fluorescence at different wavelengths. This allows multiple targets to be captured at the same test line but distinguished by an optical reader, significantly increasing the information density per unit area (Kakkar *et al.* 2024). However, its application in the field is limited due to the necessity of sophisticated optical hardware to deconvolute overlapping emission spectra (W. He *et al.* 2024).

The representative multiplexing strategies discussed above are also summarised in Figure 1.2.

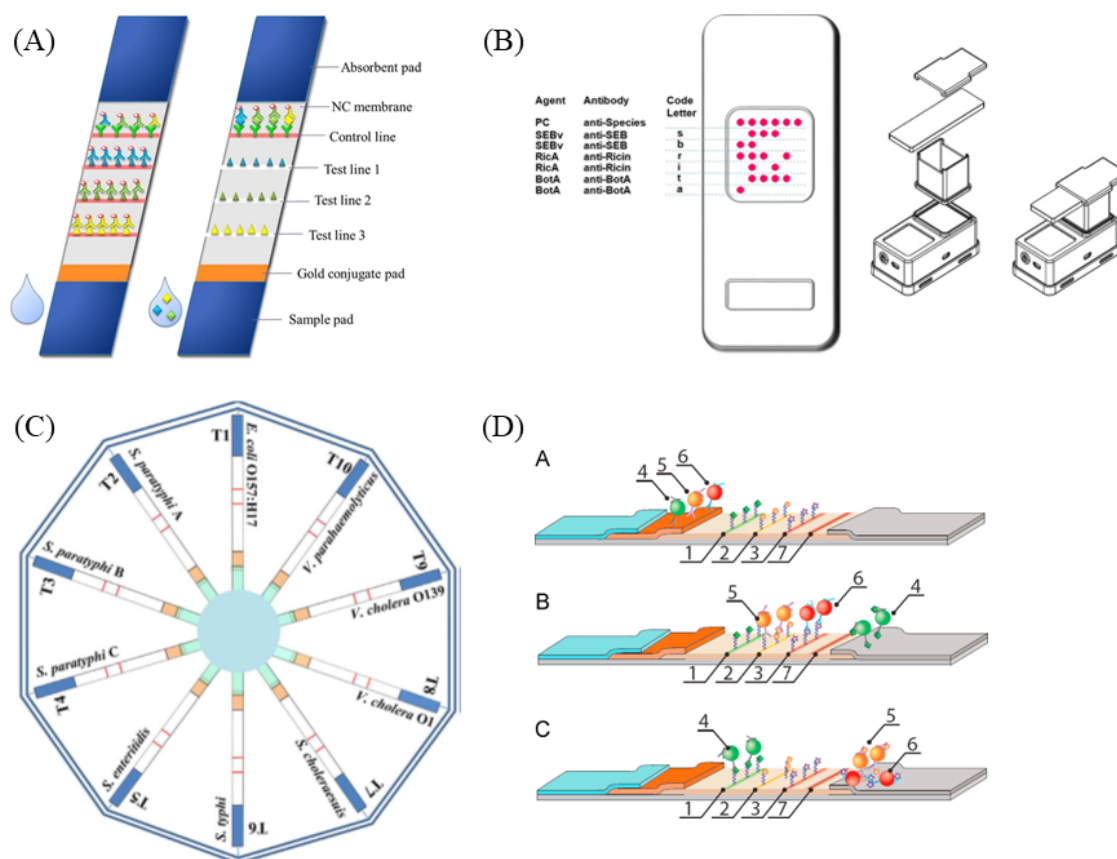


Figure 1.2: **Representative strategies for multiplexed lateral-flow strip design.** (A) Conventional multi-line lateral-flow immunoassay format for simultaneous detection of pyraclostrobin, myclobutanil and kresoxim-methyl residues, illustrating sequential test lines along a single flow path. Adapted with permission from L. Lin *et al.* 2022. Copyright 2022 Elsevier Ltd. (B) Braille-patterned dot-array lateral-flow format for multiplex biothreat-agent detection, reproduced without modification from Hofmann *et al.* 2021 under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International Licence. (C) Ten-channel lateral-flow disc for multiplex detection of foodborne pathogens, illustrating a multi-channel architecture in which separate lateral-flow strips are arranged radially within a single disc format. Adapted from Y. Zhao *et al.* 2016 under the terms of the Creative Commons Attribution 4.0 International Licence. (D) Multicolour quantum-dot "traffic-light" immunochromatographic format for simultaneous antibiotic detection, illustrating spectral/colour-encoded multiplexing using distinguishable reporter labels. Adapted with permission from N. Taranova *et al.* 2015. Copyright 2015 Elsevier B.V.

1.2.2 Thermal Management Strategies for Isothermal Amplification

Precision thermal control remains a fundamental prerequisite for nucleic acid amplification. While isothermal methods such as Loop-mediated Isothermal Amplification (LAMP) and Recombinase Polymerase Amplification (RPA) eliminate the necessity for rapid thermal cycling, they nonetheless require the maintenance of stable elevated temperatures (typically 60–65 °C for LAMP) for extended durations to ensure enzymatic efficiency (Kulkarni *et al.* 2024). To address this requirement in POC settings, a diverse array of heating strategies has been developed, generally categorised into active electronic feedback systems, exothermic chemical reactions, and field-induced non-contact methods.

Active Electronic Heating Systems

The most established paradigm in portable diagnostics utilises Joule heating, where electrical current is passed through a resistive element (typically metal thin films or ceramics) to generate heat ($Q = I^2R$). To ensure precision, these systems predominantly rely on closed-loop feedback mechanisms, where thermistors monitor the reaction temperature in real-time and modulate power input via Proportional-Integral-Derivative (PID) algorithms (ibid.). For instance, Jiang *et al.* demonstrated a microfluidic LAMP assay utilising a Printed Circuit Board (PCB) with patterned copper traces; their system successfully maintained a constant 63 °C, validated against commercial thermal cyclers (K. P. Jiang *et al.* 2024). However, despite their precision, the reliance on active feedback necessitates complex peripheral electronics (sensors and microcontrollers) and substantial power consumption. This increases both the device footprint and fabrication cost, rendering such systems less compatible with the disposable requirement of rapid diagnostics (Kulkarni *et al.* 2024).

Chemical and Exothermic Strategies

In pursuit of truly instrument-free operation, researchers have explored chemical heaters that generate heat through exothermic reactions, such as the hydrolysis of magnesium-iron alloys or the hydration of calcium oxide (CaO). To mitigate the transient nature of these reactions, they are frequently coupled with Phase Change Materials (PCMs), which clamp the temperature at their melting point by absorbing excess energy as latent heat (Mauk *et al.* 2024). A notable implementation by Li's group featured an Energetic Phase Change Material (EPCM) heater; upon

water activation, the magnesium-iron alloy generated sufficient heat to melt the PCM, which subsequently sustained a temperature of 65 °C for over 60 minutes, enabling a colourimetric SARS-CoV-2 assay (R. J. Li *et al.* 2021). Nevertheless, reviewers highlight significant limitations regarding the precision of these systems, noting that reaction rates are difficult to modulate once initiated. Furthermore, the generation of caustic chemical waste presents disposal challenges in clinical environments (Mauk *et al.* 2024).

Field-Induced and Optical Heating

Beyond contact-based methods, non-contact strategies utilising electromagnetic or optical fields have been investigated to reduce thermal mass. Magnetic induction heating dissipates eddy currents induced in conductive materials by an alternating magnetic field. Researchers exploited this principle using a 50 kHz electromagnetic coil to induce rapid heating (14.92 °C/s) within a copper-lined reaction chamber (Xie, Q. Jiang, *et al.* 2024). Alternatively, photothermal approaches leverage the Localised Surface Plasmon Resonance (LSPR) of nanomaterials. Ahn *et al.* dispersed gold nanoshells within a PCR mixture to absorb near-infrared light, achieving rapid thermal cycling suitable for quantitative PCR (Ahn *et al.* 2024). While these methods offer rapid heating rates, they introduce significant complexity: magnetic induction requires bulky high-frequency power supplies, which can generate electromagnetic interference and noise and complicate system integration (Xie, Zong, *et al.* 2025), while photothermal agents can quench fluorescence signals and require precise optical alignment (Phuong *et al.* 2026).

The Potential of Self-Regulating Materials

Collectively, the literature reveals a challenge in POC thermal management: active electronic systems offer precision at the cost of complexity, while chemical heaters offer simplicity at the cost of control. A promising alternative that bridges this gap is the use of Positive Temperature Coefficient (PTC) materials. These composites exhibit a non-linear increase in electrical resistance as temperature rises, a phenomenon caused by the thermal expansion of the polymer matrix disrupting the conductive pathways (J. Li *et al.* 2025). As demonstrated by Pardy *et al.*, this intrinsic physical property acts as a passive feedback loop, allowing the heater to self-regulate at a specific setpoint (e.g., 63 °C) without the need for external sensors or PID controllers (Pardy *et al.* 2017). This sensor-free capability presents a unique opportunity to develop heating elements that are both precise and sufficiently simple for disposable applications.

Representative thermal-management strategies for portable nucleic-acid amplification are compared in Figure 1.3. Active electronic systems use resistive heaters, sensors and feedback control to maintain the reaction temperature. Chemical heaters generate heat through exothermic reactions and often rely on insulation or phase-change materials to buffer the temperature. Optical and photothermal systems convert light into heat, enabling rapid non-contact thermal actuation but requiring optical alignment and additional hardware. Self-regulating PTC heaters provide a passive feedback mechanism through the intrinsic temperature-dependent resistance of the heating material, reducing the need for external temperature-control electronics.

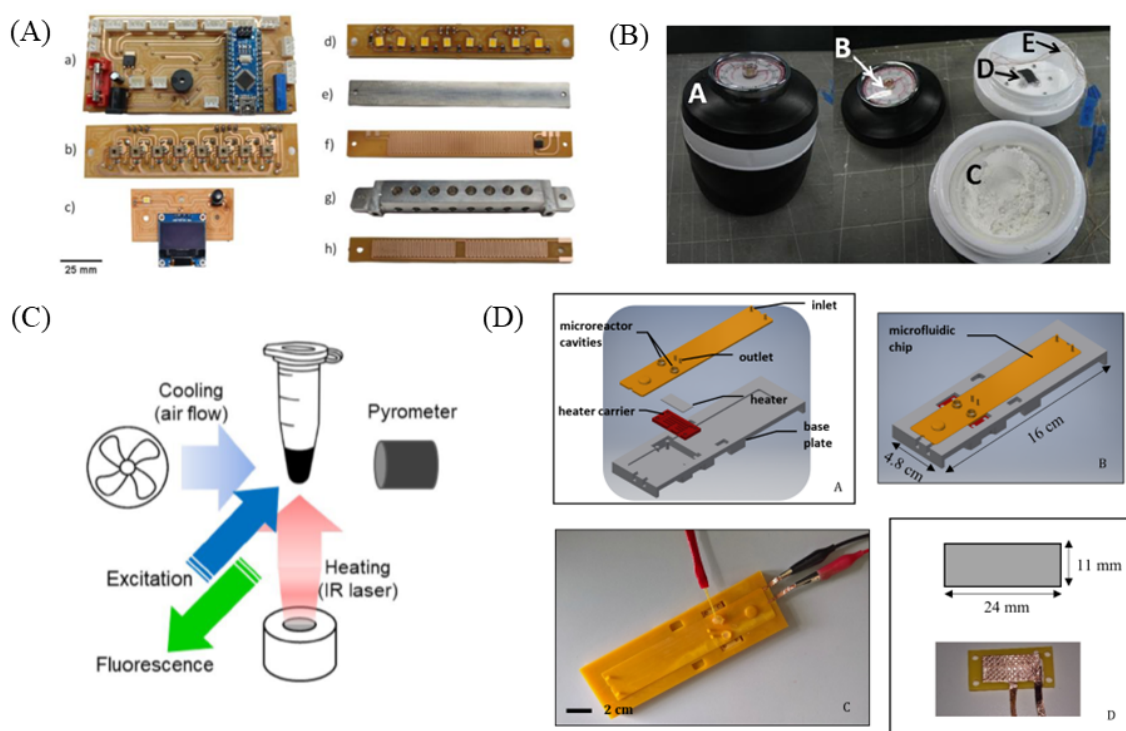


Figure 1.3: **Representative thermal-management strategies for nucleic-acid amplification.** (A) Active electronic heating and optical readout system based on Arduino-controlled RT-LAMP instrumentation, including the microcontroller, heater boards, temperature sensor, optical components and aluminium thermal blocks. Adapted from Camargo *et al.* 2024. © 2024 by the authors. Licensee MDPI, Basel, Switzerland. Distributed under the terms and conditions of the Creative Commons Attribution 4.0 International Licence. (B) Electricity-free chemical heating device for nucleic-acid amplification, using an insulated container and calcium oxide chamber to generate heat without an external power supply. Adapted from LaBarre *et al.* 2011. © 2011 LaBarre *et al.* Distributed under the terms of the Creative Commons Attribution License. (C) Photothermal quantitative PCR setup using Au nanoshells as photothermal converters, with optical excitation, active cooling, fluorescence monitoring and non-contact temperature measurement. Adapted from Ahn *et al.* 2024 under the terms of the Creative Commons Attribution 4.0 International Licence. (D) Integrated self-regulating resistive heating setup for isothermal nucleic-acid amplification in a lab-on-a-chip device, showing the microfluidic chip, heater carrier, integrated PTC heating element and assembled battery-powered test configuration. Adapted from Pardy *et al.* 2017. © 2017 Pardy *et al.* Distributed under the terms of the Creative Commons Attribution License.

1.2.3 Paper as a Functional Matrix for Molecular Diagnostics

The selection of a substrate dictates not only the fluidic driving mechanism but also the overall complexity and manufacturability of a diagnostic device. Traditional microfluidic platforms are typically fabricated from polymers such as Polydimethylsiloxane (PDMS), Polymethyl Methacrylate (PMMA), or Polyvinyl Chloride (PVC). These materials offer smooth, non-porous surfaces that allow for precise volume handling and defined channel geometries. However, their inherent hydrophobicity necessitates external active pumping systems (e.g., syringe pumps) or complex surface treatments to drive fluid flow, which inevitably increases the device's footprint and dependence on peripheral instrumentation (Reynolds *et al.* 2023).

In contrast, paper-based substrates leverage intrinsic hydrophilicity to transport fluids via capillary action (H. Lim *et al.* 2019). This pump-free passive wicking capability is the defining advantage of Microfluidic Paper-based Analytical Devices (μ PADs), eliminating the need for external power and bulky equipment (H. Liu *et al.* 2026). Furthermore, paper is biodegradable, cost-effective, and offers a high surface-area-to-volume ratio favourable for reagent immobilisation (Panigrahy *et al.* 2025).

Functional Applications: Storage and Reaction

Beyond fluid transport, paper is increasingly engineered to function as an active matrix for biochemical preservation and reaction. A critical application is the long-term stabilisation of biochemical reagents, enabling cold-chain-free storage. The porous structure of paper supports the lyophilisation (freeze-drying) of enzymes and primers. For instance, Kumar *et al.* demonstrated that isothermal amplification reagents stored in glass fibre pads with a trehalose-dextran sugar matrix retained enzymatic activity for prolonged periods at room temperature by restricting molecular mobility in a glass-like state (Kumar *et al.* 2020).

Recent trends have also moved towards performing nucleic acid amplification (e.g., LAMP or RPA) directly within the paper matrix, rather than eluting the sample for downstream reaction (Xu *et al.* 2016, Seok *et al.* 2017). For example, Sen *et al.* reported an integrated paper-based nucleic acid testing platform where both LAMP amplification and CRISPR detection occur entirely on the paper membrane (Sen *et al.* 2024).

Fluidic Manipulation Strategies

Controlling fluid timing and direction in a porous medium without mechanical valves requires manipulating the permeability and wettability of the substrate. The most prevalent technique for defining 2D fluidic channels is wax printing, where printed wax is melted to penetrate the fibre network, forming inexpensive hydrophobic barriers (Carrilho *et al.* 2009). Alternatively, laser cutting can physically remove material to create hollow channels or air gaps that guide flow with higher resolution, though this risks local carbonisation of the paper substrate (Modha *et al.* 2021, Kalish *et al.* 2020).

To increase functional density, researchers have utilised origami (folding) or stacking methods to create “3D μ PADs”. In these architectures, fluid flows vertically between layers, allowing for complex, multi-step operations (such as DNA extraction followed by amplification) to be executed sequentially by simply folding the device (Reboud *et al.* 2019). To automate these multistep reactions, passive valving mechanisms are employed to pause and release flow. These include dissolvable sugar bridges that degrade upon fluid contact to open a connection (Houghtaling *et al.* 2013), or thermally actuated wax valves that melt upon heating to allow fluid to pass (Phillips *et al.* 2016). These mechanisms enable the programmable delivery of reagents without external actuators, a critical requirement for integrated sample-to-answer devices.

1.2.4 Comparative Review of Existing Paper-based Platforms

The rapid expansion of the field has yielded a diverse array of paper-based and hybrid microfluidic devices for nucleic acid testing. These platforms can be broadly categorised based on their thermal management strategies and degree of integration. While the ultimate goal is to satisfy the WHO REASSURED criteria, a critical evaluation of recent literature reveals that most existing solutions compromise on either equipment independence or analytical performance.

Table 1.1 summarises a selection of representative devices reported in recent years. It highlights the diversity in amplification chemistries (LAMP, RPA, HDA) and the specific engineering trade-offs adopted by different research groups.

Table 1.1: Comparative review of representative paper-based molecular diagnostic platforms reported in recent literature. The table highlights the diversity in amplification chemistries and the trade-offs associated with different thermal management strategies.

Target Pathogen	Method	Heating Strategy	Detection	Time	Key Limitation / Ref
<i>Plasmodium</i> (Malaria)	LAMP	Hot Plate / Oven	Lateral Flow (Origami)	45 min	Complex manual folding and fluid actuation required (Reboud <i>et al.</i> 2019)
<i>E. coli</i> O157:H7	RPA	Self-Heating (Calcium Oxide)	Lateral Flow Strip	25 min	Chemical heating lacks the precision of electronic feedback loops (Feng <i>et al.</i> 2024)
SARS-CoV-2	RT-LAMP	Resistive Heater (USB-powered PCB)	Fluorescence (Smartphone)	55 min	Tethered to external power (USB); high power consumption (K. P. Jiang <i>et al.</i> 2024)
<i>Vibrio vulnificus</i> , <i>Salmonella</i>	LAMP	Resistive Heater (USB-powered)	Lateral Flow Strip	30 min	Requires continuous USB power connection; not fully instrument-free (H. P. Kim <i>et al.</i> 2025)
<i>Salmonella typhimurium</i>	tHDA	Self-Regulating PTC Heater	Lateral Flow (Sliding)	60 min	Manual sliding of paper components required to transfer reagents (R. Tang <i>et al.</i> 2017)
<i>Chlamydia trachomatis</i>	LAMP	Self-Regulating PTC Heater	Fluorescence	25 min	Dependent on battery power; sensor-free control demonstrated (Pardy <i>et al.</i> 2017)
Zika Virus	RT-LAMP	Chemical Heater (Mg-Fe + PCM)	Colourimetric (Leuco dye)	40 min	Requires large volume of PCM; generates chemical waste (J. Song <i>et al.</i> 2016)
SARS-CoV-2	RT-LAMP	Chemical Heater (CaO + PCM)	Colourimetric	60 min	Single-use heater is bulky and creates disposal issues (R. J. Li <i>et al.</i> 2021)
Influenza A (H1N1)	RT-LAMP	External Heat Block	Lateral Flow Strip	45 min	Not integrated; paper must be incubated in separate equipment (Rodriguez <i>et al.</i> 2015)

A systematic analysis of these platforms reveals a persistent technological gap. The majority of paper-based solutions still rely on external laboratory equipment, such as hot plates or water baths, to drive the amplification reaction (Reboud *et al.* 2019, Rodriguez *et al.* 2015). While these devices successfully demonstrate the compatibility of paper with molecular assays, they do not fulfil the requirement for instrument-free operation.

Conversely, devices that attempt fully autonomous operation often struggle with thermal precision. Chemical heaters offer an electricity-free solution but are prone to environmental fluctuations and lack the ability to actively regulate temperature without bulky Phase Change Materials (PCMs) (J. Song *et al.* 2016). Electronic heaters provide the necessary precision but typically require complex feedback circuits and continuous power delivery (K. P. Jiang *et al.* 2024), which increases device cost and complexity. Consequently, there remains a clear need for a thermal management strategy that bridges this divide—offering the precision of electronic systems with the simplicity of self-regulating materials.

1.3 Problem Statement and Research Gaps

Despite the substantial advancements in POC diagnostic technologies over the past decade, a fully integrated, instrument-free platform that satisfies the World Health Organisation’s RE-ASSURED criteria (Real-time connectivity, Ease of specimen collection, Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable) remains elusive (Land *et al.* 2018). While LFS have successfully decentralised antigen testing, they frequently lack the analytical sensitivity required for early-stage infection detection. Conversely, molecular techniques like NAATs offer superior sensitivity but have historically been limited to centralised laboratories due to complex thermal and fluidic requirements (Chakraborty 2024). Although the translation of isothermal amplification methods (e.g., LAMP, RPA) to paper-based microfluidics has shown promise in bridging this divide, existing solutions are hindered by three fundamental engineering barriers that compromise their deployment in resource-limited settings.

Gap 1: The Thermal Management Conflict

A critical barrier to the decentralisation of molecular diagnostics is the inherent trade-off between thermal precision and device simplicity. Isothermal amplification chemistries, such as LAMP, require a strictly maintained temperature window (typically 60–65 °C) to ensure optimal enzymatic turnover and prevent non-specific amplification (Kulkarni *et al.* 2024). Current thermal management strategies predominantly fall into two polarised categories:

- **Active Electronic Systems:** Devices utilising resistive heaters and Proportional-Integral-Derivative (PID) controllers offer high precision. However, they necessitate complex peripheral circuitry, temperature sensors, and significant power consumption, thereby increasing the device footprint and cost (Z. E. Jeroish *et al.* 2021).
- **Passive Chemical Heaters:** Electricity-free exothermic reactions (e.g., Mg-Fe alloys or CaO) enable instrument-free operation but suffer from poor modulation. These systems are prone to thermal runaway or rapid decay and often require bulky Phase Change Materials (PCMs) to buffer temperature spikes (Mauk *et al.* 2024).

Consequently, there is a distinct lack of a “sensor-free” heating mechanism that possesses intrinsic self-regulation capabilities to bridge the gap between the precision of electronic feedback loops and the simplicity of chemical sources.

Gap 2: The Multiplexing Limit in Lateral Flow

While respiratory infections often present with overlapping symptoms caused by distinct pathogens (e.g., SARS-CoV-2, Influenza A/B, RSV), traditional paper-based platforms are severely constrained in their multiplexing capacity. The conventional LFA architecture, which arranges capture antibodies in sequential lines perpendicular to the flow, is spatially limited by membrane length and capillary hydrodynamics (Hofmann *et al.* 2021).

As the number of target lines increases, the strip length must increase, leading to flow deceleration and non-uniform reagent delivery, where closely spaced signals become indistinguishable. Furthermore, sequential capture zones induce a hook effect or reagent depletion phenomenon, wherein high-concentration upstream targets consume the majority of colourimetric

reporters (e.g., gold nanoparticles), resulting in false negatives for downstream targets (Pedreira-Rincón *et al.* 2025). A high-density, low-resistance spatial arrangement capable of simultaneous multi-analyte detection without hydrodynamic compromise is currently missing from standard LFA design libraries.

Gap 3: The Integration Challenge

The seamless integration of “in-situ amplification” with flow control within a porous matrix remains a significant engineering challenge. Combining DNA extraction, amplification, and lateral flow detection into a single “sample-to-answer” workflow requires precise temporal control to ensure the amplification reaction reaches completion (typically 20–30 minutes) before the sample wicks into the detection zone (Sritong *et al.* 2023).

Current paper-based microfluidic devices often rely on complex manual interventions to achieve this timing, such as sliding mechanisms, user-actuated buttons, or intricate origami folding steps (Xu *et al.* 2016, Connelly *et al.* 2015). These manual operations not only reduce user-friendliness but also introduce variability and the risk of contamination in field settings (Chakraborty 2024). There remains a need for passive, autonomous valving mechanisms that can synchronise biochemical reaction times with fluidic transport in a porous medium without relying on external pumps or complex user manipulation.

1.4 Aim and Objectives

1.4.1 Primary Aim

The overarching aim of this thesis was to develop and evaluate the engineering principles required for a low-cost, paper-based nucleic-acid amplification and lateral-flow detection platform for respiratory-virus targets. The work focused on three interlinked technological functions that can support instrument-free or instrument-minimal molecular diagnostics: passive thermal regulation for isothermal amplification, compact visual multiplex readout, and passive synchronisation of amplification with downstream capillary-flow detection.

The work was designed as an analytical and engineering proof of principle using synthetic DNA templates. It did not aim to deliver a clinically validated respiratory-virus diagnostic test. Instead, it aimed to determine whether the key thermal, biochemical, fluidic, and lateral-flow functions could be designed and combined in a low-cost paper-based device architecture.

1.4.2 Specific Objectives

To achieve this aim, the research is structured around five specific technical objectives:

- **To establish design principles for a paper-compatible, self-regulating PTC-ink heater for LAMP thermal management:** This objective focused on the relationship between PTC-ink heater geometry, electrical resistance, power input, and liquid-phase temperature control. The intended technological outcome was a set of practical design criteria for using high-resistance PTC ink as a passive heating material within the LAMP temperature range of approximately 60–65 °C.
- **To determine the material requirements for using paper as both an amplification matrix and an amplicon-release medium:** This objective focused on the compatibility between porous substrates, LAMP chemistry, and downstream lateral-flow detection. The intended scientific outcome was to identify the substrate properties required to support on-paper amplification while allowing amplified DNA to be released efficiently into the detection strip.
- **To establish a compact multiplex nucleic-acid lateral-flow format that reduces the limitations of conventional multi-line strips:** This objective focused on the spatial organisation of capture zones in multiplex lateral-flow detection. The intended technological outcome was a lateral-flow architecture capable of improving multi-target amplicon readout while reducing spatial crowding, downstream reagent depletion, and signal interference associated with line-based formats.
- **To define the design parameters governing passive, thermally actuated fluidic timing in paper-based channels:** This objective focused on wax-gate actuation as a mechanism for delaying capillary transport until completion of the amplification step. The intended technological outcome was a passive timing strategy that links the thermal conditions required for LAMP with controlled downstream release into the lateral-flow detection zone.

- **To assess the integration constraints and analytical detection performance of a proof-of-principle paper-based LAMP–lateral-flow device using synthetic DNA:** This objective focused on the compatibility of the heating, amplification, wax-gate, and lateral-flow modules when combined into a single device workflow. The intended outcome was to evaluate device-level feasibility and observed analytical limit of detection under controlled synthetic-DNA conditions, while recognising that clinical sample preparation and real-sample validation remained outside the scope of the thesis.

1.5 Scope of the Thesis

This thesis focused on the engineering development and analytical proof-of-principle evaluation of a paper-based LAMP–lateral-flow diagnostic platform. All nucleic-acid amplification and detection experiments were performed using synthetic DNA templates or amplicons generated from synthetic DNA templates. The synthetic templates represented respiratory-virus targets, including SARS-CoV-2, Influenza A, Influenza B, RSV A, and RSV B.

No clinical respiratory samples were processed in this thesis. No attempt was made to recover, lyse, extract, or purify nucleic acids from real biological specimens. The work also did not include viral RNA extraction, reverse transcription from clinical RNA, or validation against patient-derived nasopharyngeal swabs. Therefore, the analytical performance reported in this thesis refers to the detection of synthetic DNA under laboratory conditions.

The final integrated prototype described in Chapter 5 was evaluated as a single-target proof-of-principle system using Influenza B synthetic DNA. Although Chapter 4 developed a five-target dot-array lateral-flow strip, the final Chapter 5 integrated device did not yet incorporate the full five-target dot-array readout. The transfer of the multiplex dot-array strip into the final integrated device architecture remains a future implementation step. This distinction is also consistent with the current Future Work section, where the thesis already identifies integration of the Chapter 4 dot-array into the final device as a short-term next step.

The scope of the thesis is therefore best defined as the development of enabling device principles for future paper-based molecular diagnostics: passive printed thermal regulation, compact multiplex lateral-flow readout, on-paper amplification and release, and passive time-programmed fluidic transfer.

1.6 Statement of Original Contribution

This thesis was carried out within an interdisciplinary research environment involving nucleic-acid amplification, lateral-flow diagnostics, printed electronics, and paper-based microfluidics. Unless otherwise stated below, all experimental work, device fabrication, optimisation, data collection, data analysis, interpretation, figure preparation, and thesis writing were carried out by the candidate. The following collaborative inputs and external sources contributed to specific parts of the thesis:

- The LAMP primer sets used for SARS-CoV-2, Influenza A, Influenza B, RSV A, and RSV B were obtained from previously published literature, as referenced in Chapter 2.
- The LAMP reaction formulations and primer ratios used for the multiplex Influenza and RSV assays were provided by Dr Pawel Jajesniak.
- The micro-scale interdigitated electrode devices described in Chapter 3, Section 3.1.11 were fabricated and characterised with assistance from Professor Jeff Kettle's group, University of Glasgow.

All other parts of the work presented in this thesis, including the development and optimisation of the paper-based PTC heater, the multiplex lateral-flow strip design and testing, the on-paper amplification experiments, the wax-gate characterisation, the integrated device development, and the analytical testing of the final prototype, were performed by the candidate.

Chapter 2

Materials and Methods

This chapter details the experimental design and methodologies and describes the procedures performed and the equipment used.

2.1 LAMP reagents

2.1.1 Master Mixes and Buffers

Loop-mediated isothermal amplification (LAMP) experiments were conducted using two isothermal master mixes—ISO-001 for SARS-CoV-2 detection and ISO-004 for Influenza A/B and RSV A/B—both obtained from OptiGene Ltd. (Horsham, England).

Nuclease-free water (NFW) was used to prepare all aqueous solutions. Phosphate buffer saline (PBS) and Tris-EDTA (TE) buffer were used as the running buffer for lateral flow strips. All reagents were purchased from Sigma-Aldrich (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany).

2.1.2 Synthetic DNA

Influenza A gene DNA (248 bp), Influenza B gene DNA (237 bp), RSV A gene DNA (269 bp), RSV B gene DNA (242 bp) and COVID gene DNA (500 bp) were purchased from Eurofins Scientific (Eurofins Scientific, Luxembourg) (Sequences provided in Appendix A). In DNA diagnostic assays, the limit of detection (LOD) is typically expressed in copies per microlitre (copies/ μL). In our project, each reaction used 5 μL of DNA sample. Therefore, it was necessary to convert the DNA concentration from ng/ μL to copies/5 μL (copies/reaction) to accurately determine the LOD. The number of DNA copies was calculated using the following

formula:

$$\text{Number of copies} = \frac{dsDNA \text{ mass [ng]} \times Avogadro's \text{ number [number/mole]}}{dsDNA \text{ length [bp]} \times (1 \times 10^9) \text{ [ng/g]} \times 650 \text{ [g/mole of bp]}} \quad (2.1)$$

with *Avogadro's number* = 6.022×10^{23} molecules/mole and 650 Daltons were used as the average weight of a base pair (bp).

Initially, all DNA templates were at a concentration of 300 ng. Through serial dilutions with nuclease-free water, a stock concentration of 0.02 ng/ μ L was prepared, equating to 0.1 ng/reaction. Using the formula above, this concentration can be converted into 1×10^7 copies/reaction. The calculation results are shown in Table 2.1. This stock solution was further diluted as needed for subsequent experiments.

Table 2.1: **Conversion of DNA concentration from 0.02 ng/ μ L to 1×10^7 copies/reaction.**

DNA template	bp	DNA copies per 0.1 ng	Volume of template[μ L]	Volume of nuclease-free water[μ L]
Inf A	248	3.941E+08	25.37	974.63
Inf B	237	4.124E+08	24.25	975.75
RSV A	269	3.634E+08	27.52	972.48
RSV B	242	4.039E+08	24.76	975.24
COVID	500	1.955E+08	51.15	948.85

2.1.3 Primers

The primer sequences for LAMP detection of influenza A/B, RSV A/B, and COVID are provided in Table 2.2. All primers were purchased from Eurofins Scientific. For each LAMP reaction, a 5 μ L primer mix was prepared, with all primers pre-diluted to a stock concentration of 20 μ M. The specific volumes of each primer used in the mix are detailed in Table 2.3. Introducing the labelled primers in LAMP reactions, such as the *LF*TAM* and *LB*BIO* primers in Inf A assay, reduced amplification efficiency (Altattan *et al.* 2024). Therefore, we utilised a 1:1 ratio of labelled to unlabelled primers to balance the performance.

Table 2.2: **Primer sequences of LAMP assays.**

Species	Oligos	Sequence (5'-3')	Source
Influenza A	F3_1	5'-GACTTGAAGATGTCTTTGC	Nakauchi <i>et al.</i> 2010
	F3_2	5'-GACTGGAAAAGTGTCTTTGC	
	B3_1	5'-TRTTATTTGGGTCTCCATT	
	B3_2	5'-TRTTGTTTGGGTCCCCATT	
	FIP	5'-TTAGTCAGAGGTGACARRATTGCAGATCTTGAGGCTCTC	
	BIP	5'-TTGTKTTTCACGCTCACC GTGTTTGGACAAAGCGTCTACG	
	LF*TAM	5'-TAMRA-GTCTTGTCTTTAGCCA	
	LB*BIO	5'-Biotin-CMAGTGAGCGAGGACTG	
Influenza B	F3	5'-GCAACCAATGCCACCATA	Takayama <i>et al.</i> 2019
	B3	5'-TTCTCTCTTCAAGRGACATC	
	FIP*BIO	5'-Biotin-TAGTCAAGGGCYCTTTGCCACTTTGAAGCAGGAATTCTGGA	
	BIP	5'-CAAGACCGCCTAAACAGACTAAACTTTTACTTTCAGGCTCACTT	
	LF*FITC	5'-FITC-TGAAAGYCTTTCATAGCAC	
RSV A	LB	5'-CAAGAATAAAGACTCACAAC	Mahony <i>et al.</i> 2013
	F3	5'-GCTGTTCAATACAATGTCCTAGA	
	B3	5'-GGTAAATTTGCTGGGCATT	
	FIP	5'-TCTGCTGGCATGGATGATTGGAGACGATGATCCTGCATCA	
	BIP	5'-CTAGTGAAACAAATATCCACACCCAGCACTGCATTCTTGAGTT	
	LF*AF488	5'-Alexa Fluor 488-ACATGGGCACCCATATTGTAAG	
RSV B	LB*BIO	5'-Biotin-AGGGACCTTCATTAAGAGTCATGAT	Shirato <i>et al.</i> 2007
	F3	5'-GGGCAAATATGGAAACATACG	
	B3	5'-CCTTTGGGCGTAGAGATC	
	FIP	5'-GTTAGTGATGCAGGATCATCATCTTTTTTTGAACAAGCTTCACGAAGG	
	BIP	5'-TGGGTGCCTATGTTCCAGTTTTTGCTTCACTAGTATGTTGATGCT	
COVID	LF*DIG	5'-Digoxigenin-GTACTGAACAGCTGCTGTGTAT	Papamathaiou <i>et al.</i> 2023
	LB*BIO	5'-Biotin-GCAGACTTGCTCATAAAGAAGCTTG	
	F3	5'-TCCAGATGAGGATGAAGAAG	
	B3	5'-CAACAATTGTTTGAATAGTAG	
	FIP	5'-AGAGCAGCAGAAGTGGCAGGTGATTGTGAAGAAGAAGAGT	
	BIP	5'-TCAACCTGAAGAAGAGCAAGAAGTCTGATTGTCCTCACTGCC	
	LF*DNP	5'-Dinitrophenyl-CTCATATTGAGTTGATGGCTC	
	LB*BIO	5'-Biotin-CAAACCTGTTGGTCAACAAGAC	

For multiplexed detection, our goal was to amplify multiple targets simultaneously within a single device. Therefore, we aimed to decrease the number of reactions by combining several reactions in one pot. However, including many primers in one reaction increases the likelihood of primer-dimer formation and non-specific amplification (Elnifro *et al.* 2000, Meagher *et al.* 2018). To address this, a postdoctoral researcher in our group, Pawel Jajesniak, optimised the LAMP assays for Influenza and RSV, allowing for the simultaneous amplification of two subtypes in a single reaction. The optimised reaction components are detailed in Table 2.4.

2.2 LAMP Conditions

This section details the experimental conditions of the Loop-Mediated Isothermal Amplification (LAMP) reaction required for the integrated diagnostic platform. Establishing robust amplification methods is critical to ensuring assay sensitivity and reliability before integrated into a device. To achieve this, the reaction was evaluated across three different formats, starting from standard laboratory protocols to the final paper-based implementation.

Table 2.3: **Volume of each component in primer mix for single-plex LAMP reaction (Unit: μL).**

InfA	Volume	InfB	Volume	RSVA	Volume	RSVB	Volume	COVID	Volume
F3	0.25	F3	0.25	F3	0.25	F3	0.25	F3	0.2
B3	0.25	B3	0.25	B3	0.25	B3	0.25	B3	0.2
FIP	1	FIP*BIO	0.5	FIP	1	FIP	1	FIP	1
BIP	1	FIP	0.5	BIP	1	BIP	1	BIP	1
LF*TAM	0.25	BIP	1	LF*AF488	0.25	LF*DIG	0.25	LF*DNP	0.25
LF	0.25	LF*FITC	0.25	LF	0.25	LF	0.25	LF	0.25
LB*BIO	0.25	LF	0.25	LB*BIO	0.25	LB*BIO	0.25	LB*BIO	0.25
LB	0.25	LB	0.5	LB	0.25	LB	0.25	LB	0.25
Water	1.5	Water	1.5	Water	1.5	Water	1.5	Water	1.6
Total	5	Total	5	Total	5	Total	5	Total	5

Table 2.4: **Volume of each component in primer mix for multiplexed LAMP reaction.**

Influenza	Volume(μL)	RSV	Volume(μL)
InfA F3_1	0.0625	RSVA F3	0.125
InfA F3_2	0.0625	RSVA B3	0.125
InfA B3_1	0.0625	RSVA FIP	0.5
InfA B3_2	0.0625	RSVA BIP	0.5
InfA FIP	0.5	RSVA LF*AF488	0.125
InfA BIP	0.5	RSVA LF	0.125
InfA LF*TAM	0.25	RSVA LB*BIO	0.125
InfA LB*BIO	0.25	RSVA LB	0.125
InfB F3	0.25	RSVB F3	0.125
InfB B3	0.25	RSVB B3	0.125
InfB FIP	0.875	RSVB FIP	0.5
InfB FIP*BIO	0.125	RSVB BIP	0.5
InfB BIP(40 μM)	0.5	RSVB LF*DIG	0.125
InfB LF*FITC	0.125	RSVB LF	0.125
InfB LF	0.375	RSVB LB*BIO	0.125
InfB LB	0.5	RSVB LB	0.125
Water	0.25	Water	1.5
Total	5	Total	5

First, Real-Time LAMP conditions are described, where amplification curves were monitored using a q-PCR machine to establish a standard performance. Subsequently, In-tube LAMP protocols were conducted to validate endpoint detection methods suitable for lateral flow read-out. Finally, LAMP on Paper conditions were investigated to adapt the reaction to the cellulose matrix, addressing specific challenges related to reagent stability, inhibition, and product recovery within the porous substrate.

2.2.1 Real-Time LAMP Conditions

Real-time LAMP amplification was conducted using the QuantStudio™ 3 Real-Time PCR System instrument (Applied Biosystems; Thermo Fisher Scientific Inc.). The thermal protocol consisted of 30 cycles of 1 min at 65°C, corresponding to a total reaction time of 30 minutes. Following amplification, a melt curve analysis was performed with a two-step protocol: 95°C for 15 seconds, followed by 80°C for 1 minute. To confirm the specificity of amplification, melting temperatures (T_m) were analysed using the QuantStudio™ Design & Analysis Software (Applied Biosystems). Each target amplicon consistently produced a single peak in the melt curve, corresponding to the T_m of the respective positive control, unless stated otherwise in later sections. The final reaction volume for each LAMP assay was 25 µL, comprising 5 µL of primer mix (prepared as described in 2.3), 15 µL of isothermal master mix and 5 µL of DNA template.

All assays were performed either in duplicate ($n = 2$) or triplicate ($n = 3$), as specified in the subsequent chapters. No-template controls (NTCs) were included in each run by substituting the DNA template with 5 µL of nuclease-free water (NFW). These controls served to verify the absence of non-specific amplification and ensure that no false-positive signals were observed in the absence of target DNA.

2.2.2 In-tube LAMP Conditions

When performing LAMP reactions in PCR tubes, a larger reaction volume was used to minimise the impact of evaporation during incubation. Typically, 20 or 40 individual LAMP reactions were combined into a 1.5 mL PCR tube, resulting in a total volume of 0.5 mL or 1 mL, respectively. After reagent preparation, the tubes were placed on a preheated hot plate set to 65 °C and incubated for 30 minutes.

2.2.3 LAMP on Paper Conditions

Whatman® Grade 1 filter paper and glass microfiber filters (GF) were obtained from Cytiva (GE Life Sciences, Chalfont St Giles, Buckinghamshire, UK). All the papers were prepared either as 5 mm paper discs, using a hole puncher (Biopsy punch, Kai Medical, Kai Corporation and Kai Industries Co., Ltd., Manchester, England), or as 5 mm-wide strips cut by a paper guillotine.

To perform amplification on paper substrates, a sealed chamber was required to contain both the reagents and the paper. Two approaches were employed, corresponding to the different paper formats used.

In the first method, the paper disc was placed into a PCR tube or PCR microplate well, and LAMP amplification was carried out using either a real-time PCR machine or a heat block, as described in Section 2.2.1 and 2.2.2. The second method involved fabricating a custom chamber using a laser cutter (LS3020 Desktop Laser, HPC Laser Ltd., Halifax, United Kingdom). This chamber was constructed from two 1 mm-thick layers of poly(methyl methacrylate) (PMMA), which were cut using the laser cutter (Figure 2.1).

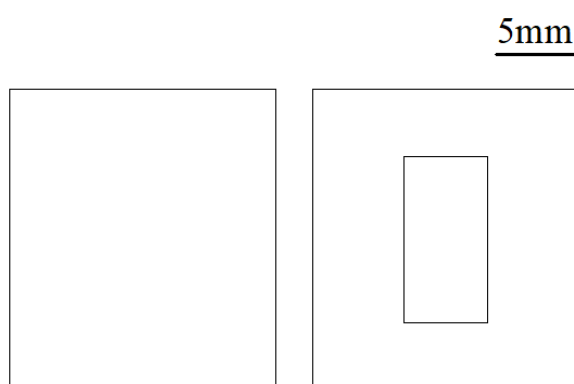


Figure 2.1: **PMMA chamber cut sheets for amplification on paper.**

A heat press (SEFA ROTEX Lite V3, SEFA S.A.S., Esperaza, France) was used to bond the two PMMA layers, forming a stable chamber structure. Once the paper strip and LAMP reagents were placed inside, the open side of the chamber was sealed with acetate films (MicroAmpTM Optical Adhesive Films), creating a closed environment to minimise evaporation during amplification.

Following amplification, it was necessary to verify the presence of amplicons on the paper. However, the paper matrix often interfered with fluorescence-based readouts, resulting in noisy or inconsistent signals when using qPCR systems or microplate readers, due to the intrinsic autofluorescence of cellulose and common paper additives (M. He *et al.* 2013). To address this, gel electrophoresis was employed as a more reliable validation method. DNA was released from

the paper by immersing the strip in gel loading buffer, allowing the amplicons to migrate out of the matrix. The samples were then run on an agarose gel (1% agarose, 1kbp ladder, running under 100 V for 1 h), and distinct DNA bands corresponding to expected product sizes were visualised under UV illumination, confirming successful amplification.

Additionally, lateral flow strips served as a complementary detection method by applying the paper directly onto the strip and developing it in a running buffer. The complete procedure for paper-based amplification is illustrated in Figure 2.2.

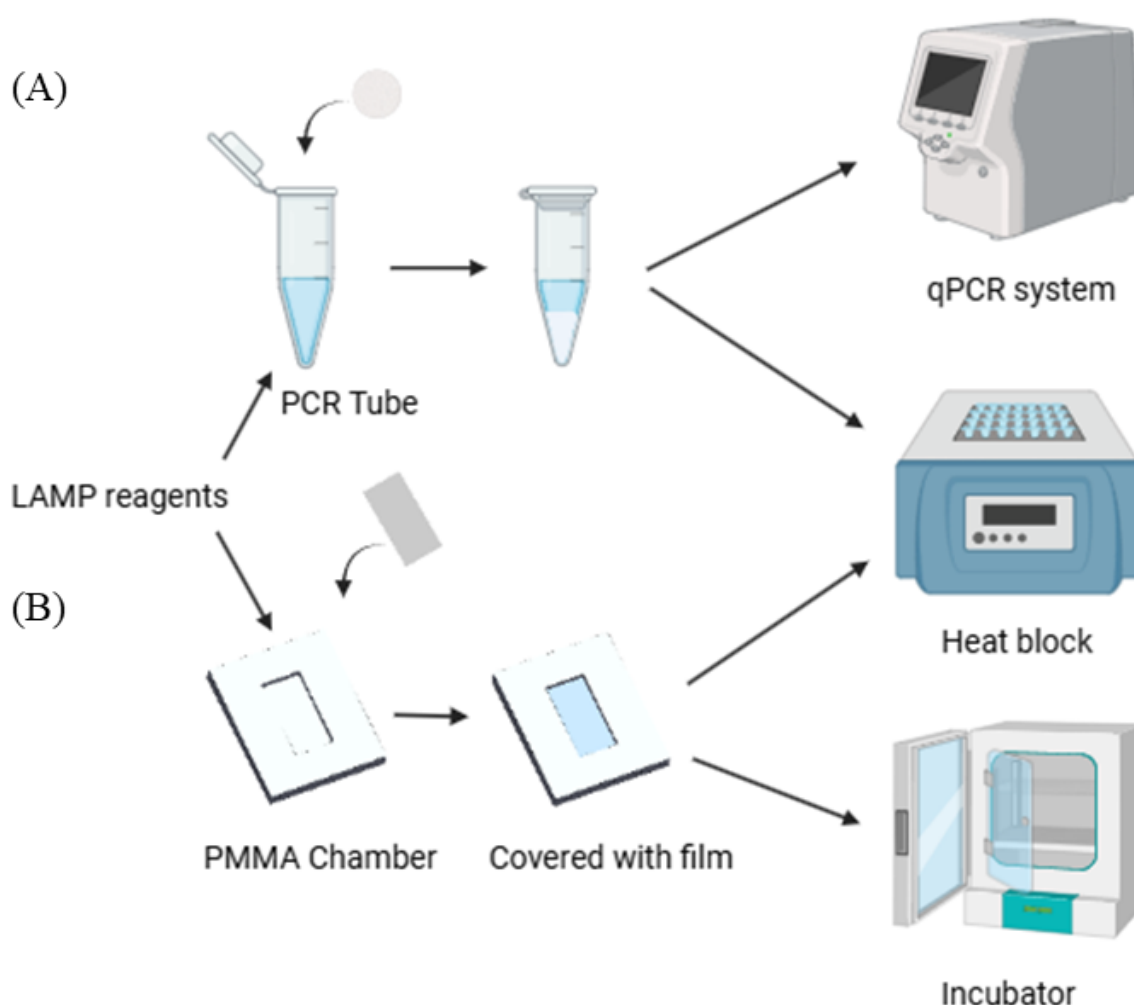


Figure 2.2: **Schematic of amplification on paper.** Two methods to conduct amplification on paper. (A) Paper amplification in PCR tubes. LAMP reagents were added after a 5 mm paper disc was placed at the bottom of the tube. The tubes were then transferred to the qPCR system or heat block for amplification for 30 minutes. (B) Paper amplification in the PMMA chamber. LAMP reagents were added after a 10 mm × 5 mm paper strip was placed in the chamber. The chamber was then sealed from the top using acetate film. The chamber was transferred to a heat block or incubator set to 65 °C for 30 minutes.

2.3 Analytical Limit-of-Detection Assessment of the LAMP and Lateral Flow Assays

To determine the analytical LoD of the LAMP assay, a ten-fold serial dilution was first performed using the Influenza B synthetic DNA template, as described in Section 2.2. For multiplex detection involving all five targets, RSV B was selected as the representative target model. Although the strip is designed to detect five pathogens, RSV B was chosen for this specific validation because its capture zone is located at the most downstream position of the dot array (distal to the sample pad).

In lateral flow assays, flow velocity decreases as the fluid flows through the membrane, and reagent availability can be compromised by non-specific adsorption along the membrane path (X. Zhao *et al.* 2024). Therefore, the RSV B zone represents the worst-case scenario for reagent transport and delivery. Successful detection at this distal position validates the flow integrity of the entire array. For this assessment, serial dilutions of synthetic RSV B DNA were tested individually on the penta-plex strips to establish the baseline sensitivity of the micro-dot format.

The evaluation was performed using synthetic DNA templates at concentrations ranging from 1×10^5 to theoretical 1 copy per reaction in LAMP. Each concentration was tested in triplicate, and detection was carried out using lateral flow strips. The limit of detection (LOD) was defined as the lowest DNA concentration that consistently yielded a visible test line in all replicates.

2.4 Fabrication of Lateral Flow Strips

2.4.1 Material and Equipment

Typically, lateral flow strips were composed of a sample pad, a conjugate pad, a nitrocellulose membrane, an absorbent pad, and a backing card. The glass fibre pad (Catalog # GFDX203000) used for sample and conjugate pads, and the cellulose pad (Catalog # CFSP223000) for the absorbent pad were bought from MilliporeSigma (Merck KGaA, Burlington, MA, USA). Two kinds of nitrocellulose membrane were purchased from MilliporeSigma (Hi-FlowTM Plus 90) and GE Life Sciences (FF120HP Plus), respectively. Six different antibodies, specifically anti-DIG antibody (Catalog # 605-680), anti-DNP antibody (Catalog # A150-117A), anti-FITC

antibody (Catalog # A150-112A), anti-TAMRA antibody (Catalog # MA1-041), anti-Alexa Fluor 488 antibody (Catalog # 710369), and anti-streptavidin antibody (Catalog # 100-4195), were purchased from Thermo Fisher Scientific (Waltham, USA). The Streptavidin Gold Conjugate (Catalog # ab186864) was sourced from Abcam PLC (Cambridge, UK). The Nucleic Acid Probes Detection Strips were bought from Ustar Biotechnologies (Hangzhou, China). The non-contact microarray dispenser (BioSpot BT600) equipped with a Pipejet nanodispenser module was acquired from BioFluidix GmbH (Freiburg, Germany). The strip cutter utilised was obtained from Antiteck Life Sciences Limited (Guangzhou, China).

2.4.2 Buffer

Nuclease-free water (NFW) was used to prepare all aqueous solutions. All reagents were purchased from Sigma-Aldrich (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) unless otherwise specified.

Different buffers were used for the manufacturing and running of lateral flow strips. Gold conjugate buffer with components: 2 mM borate at pH 7, and 10% sucrose. Blocking buffer to block the membrane after dispensing antibody solution onto the membrane: 1% BSA. Washing buffer to remove excess blocking buffer: 5 mM Na_2HPO_4 (PH7.5), and 0.05% Tween 20. Running buffer to help and accelerate the wicking process of the membrane: PBS, 0.05% Tween 20, and 5mM MgCl_2 .

2.4.3 Procedure

The lateral flow strip fabrication procedure was adapted from established industry protocols, specifically the methodologies detailed by Merck Millipore (Merck Millipore 2019). The whole fabrication process is illustrated in Figure 2.4. It begins with the deposition of capture antibodies onto the nitrocellulose membrane at certain locations. In this work, two dispensing formats were used: lines and dots. The line format represents the conventional architecture of lateral flow assays, consisting of a test line for target capture and a control line for assay validation. While robust for single-analyte detection, this configuration becomes less reliable in multiplexed assays, where multiple antibody lines placed in close proximity can lead to signal interference, competitive binding, or cross-reactivity. To solve these issues, a dot-format layout

was employed for multiplexing, allowing spatially separated micro-spot deposition and reducing the risk of antibody interference (Anfossi *et al.* 2018). The BioSpot dispensing system (Figure 2.3) was used to deposit antibodies in either format through programmable control of dispensing parameters.

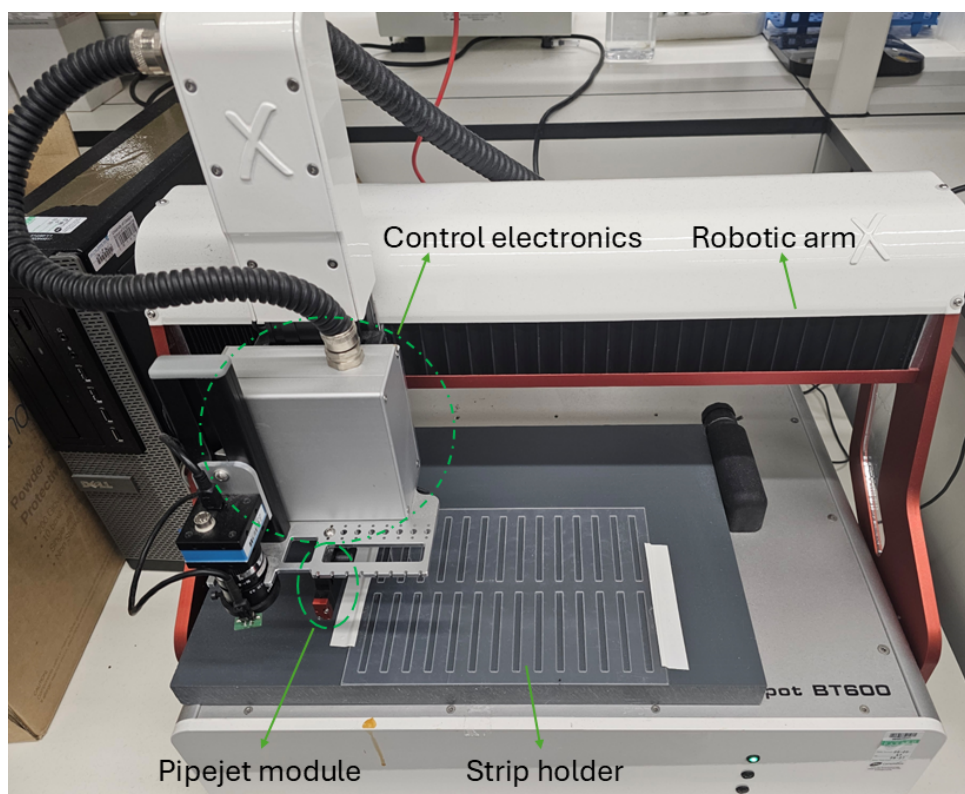


Figure 2.3: **The BioSpot BT600 antibody dispensing system (BioFluidix GmbH).** The instrument operates via control electronics that regulate the programmable XYZ-axis robotic arm to coordinate the movement of the PipeJet nanodispenser module. A dedicated strip holder is designed to secure individual lateral flow strips, ensuring precise alignment for the accurate, non-contact deposition of reagents in both line and dot formats.

Following antibody deposition, a blocking step was performed to passivate the membrane surface, stabilise immobilised antibodies, and reduce non-specific binding during the assay. A 1% BSA solution was selected due to its wide compatibility with nitrocellulose and minimal impact on downstream assay kinetics. The blocking solution was prepared in a Petri dish, and the membrane was gently immersed to allow trapped air in the porous structure to escape. Ensuring slow and uniform wetting was essential to avoid incomplete surface coverage. The membrane

remained submerged for 1 minute, after which excess BSA solution—potentially responsible for pore clogging if not removed—was rinsed away using the washing buffer described in Section 2.4.2. The membrane was immersed in the washing buffer for 5 minutes and subsequently dried in a 37 °C incubator for 2 hours.

Assembly of the full lateral flow strip was then carried out by laminating the conjugate pad, sample pad, and absorbent pad onto the treated membrane. The conjugate pad was pre-loaded with Streptavidin–Gold nanoparticles by dipping the pad into the conjugate solution and drying at 37 °C for 2 hours to ensure stable immobilisation. On the sample end, the sample pad was laminated onto the conjugate pad with a 2 mm overlap, forming a sample–conjugate–membrane sandwich structure that facilitates smooth transfer of the sample and conjugates into the membrane. On the opposite end, the absorbent pad was laminated with a 2 mm overlap to provide sufficient capillary force for fluid flow. Once fully assembled, the layered membrane was cut into 4 mm-wide strips using a strip cutter, producing individual lateral flow tests ready for evaluation.

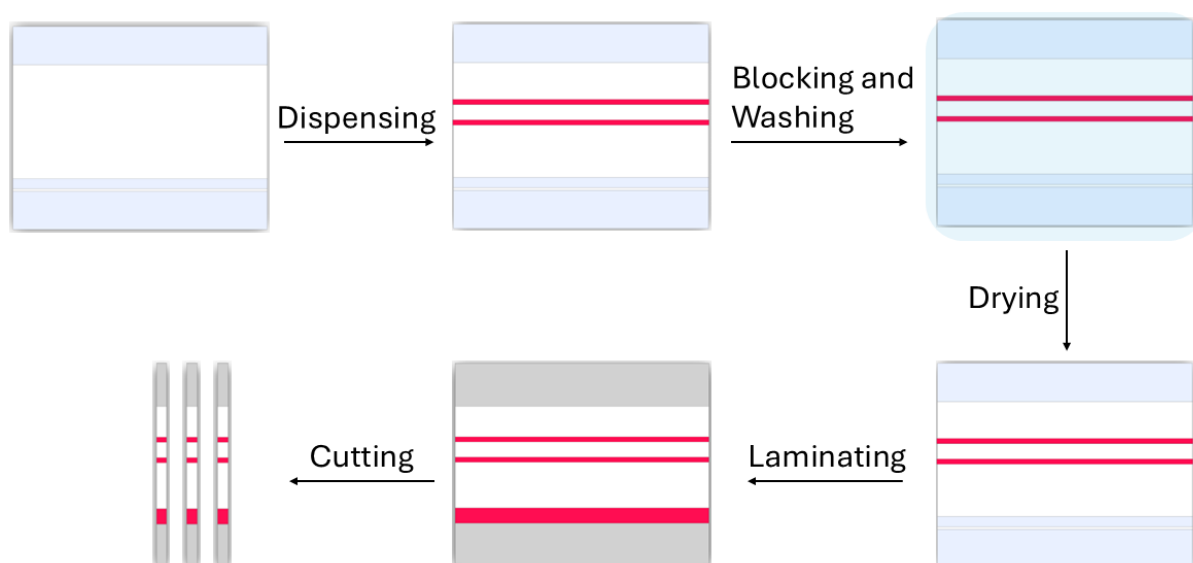


Figure 2.4: **Lateral flow strip fabrication procedure.** Firstly, the antibody solution was dispensed onto the membrane card and air-dried for 10 minutes. The card was then placed into a petri dish with blocking buffer. The blocking step lasted for 1 minute before the card was transferred into the washing buffer to remove excess BSA solution. Then, the card was placed in the incubator at 37 °C for 2 hours for complete drying. After the card was dried, the conjugate pad, the sample pad, and the absorbent pad were laminated on the membrane card. Last but not least, the whole membrane card was cut into single lateral flow strips by the strip cutter.

2.4.4 Design of Multiplexing Lateral Flow Strips

This project aimed to develop a lateral flow strip capable of simultaneous detection of five targets, i.e., a pentaplex assay. A straightforward strategy for multiplexing is to deposit multiple test lines on a single nitrocellulose membrane, each line corresponding to a different target. To explore this approach, a commercial nucleic acid detection strip (Nucleic Acid Probes Detection Strip, Ustar Biotechnologies, Hangzhou, China) was used as the base membrane. Four additional test lines were dispensed onto the strip using the BioSpot system to extend its functionality from singleplex to pentaplex detection.

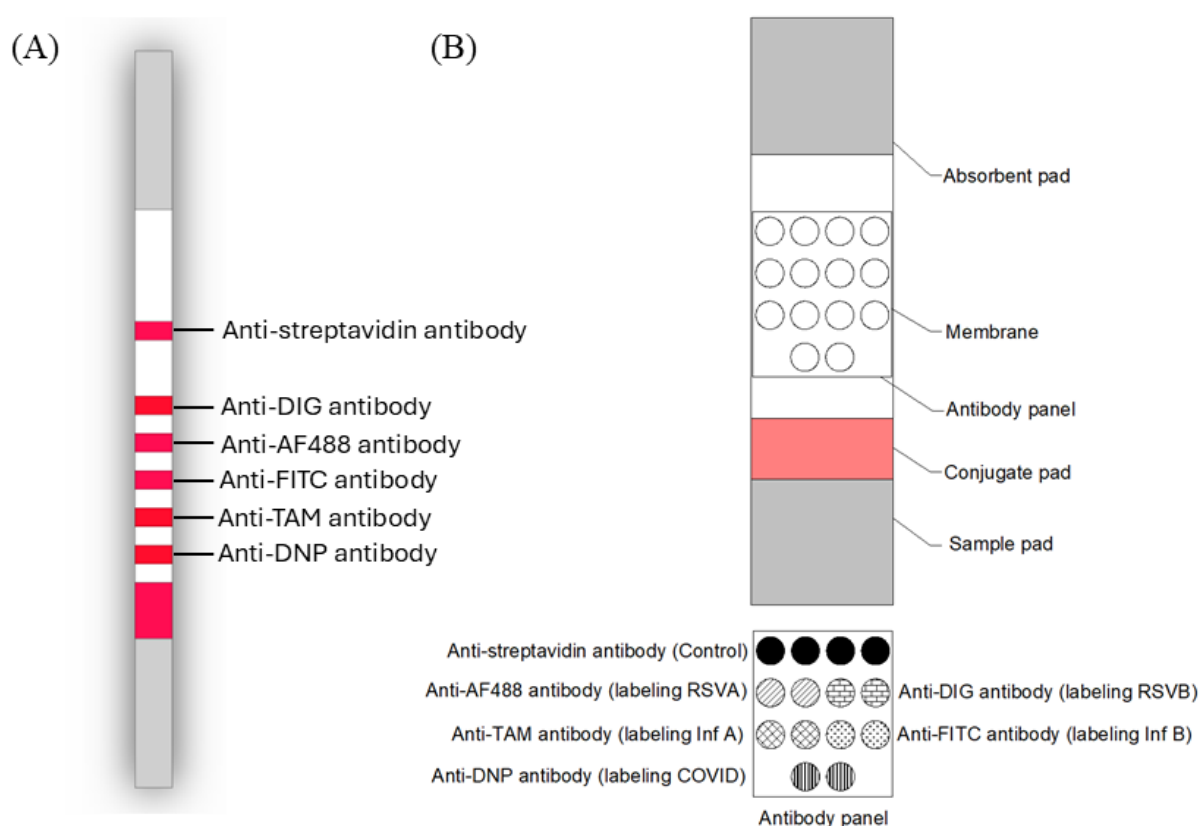


Figure 2.5: **Antibody position arrangement on multiplexing lateral flow strips.** Anti-DNP, Anti-TAM, Anti-FITC, Anti-AF488, Anti-DIG, and Anti-streptavidin antibodies were utilised to label COVID, Inf A, Inf B, RSV A, RSV B, and control, respectively. (A) Multi-line strategy where different antibodies corresponding to different targets were printed in line format and placed along the flow direction. (B) Multi-dot strategy of the lateral flow strip. The antibodies were printed in dot format, and every two dots represented a replicate of the same target.

However, the limited membrane width imposes constraints on how closely these lines can be spaced. As test lines accumulate, both signal quality and specificity can be compromised due to spatial crowding, cross-reactivity, and uneven distribution of fluid across the membrane (Anfossi *et al.* 2018). These limitations facilitated the exploration of an alternative multi-dot dispensing strategy, which offers improved spatial separation between capture regions and reduces mutual interference among antibodies. Although the dot format affords better isolation of reagents, its reduced capture area can lower the probability of target–antibody interaction. To overcome this drawback, each target was assigned two replicate dots, thereby increasing effective capture area while maintaining low cross-interaction.

For assay validation, the control zone was designed as a row of four aligned dots, ensuring reliable capture of excess Streptavidin-Gold nanoparticles and generating a distinct visual reference independent of target presence. The layout and spatial arrangement of all antibody positions—both test dots and control dots—are illustrated in Figure 2.5.

2.4.5 Optimisation of Lateral Flow Strips

To achieve reliable and reproducible signal generation in multiplex lateral flow assays, several key parameters of the lateral flow strips were investigated and optimised. These included the choice of nitrocellulose membrane, the composition of running buffers, the necessity of membrane blocking, and the spatial arrangement of capture antibodies for multiplexed detection.

Two commercially available nitrocellulose membranes—Hi-Flow Plus 90 and FF120HP Plus—were evaluated due to their different capillary flow rates and surface properties. The membranes were compared based on liquid wicking speed, signal intensity, and background noise when used in the same strip configuration. Slower-flow membranes provided longer interaction times between analytes and capture antibodies, which was beneficial for signal development in multiplex formats, while faster-flow membranes enabled shorter assay times but occasionally resulted in weaker signals or incomplete binding (P. Jia *et al.* 2023). These comparisons informed the selection of a membrane that balanced assay speed with sensitivity and reproducibility.

The composition of running buffers was also assessed, as buffer chemistry strongly influences nanoparticle stability, flow behaviour, and non-specific binding. Buffers with varying salt concentrations and surfactant content were tested to improve flow consistency and reduce background signals. In parallel, the necessity of a membrane blocking step was examined by

comparing blocked and unblocked membranes. Blocking with bovine serum albumin (BSA) was found to stabilise antibody immobilisation and reduce non-specific adsorption (Spreinat *et al.* 2025), particularly in multiplexed formats where multiple capture regions are present on the same membrane.

Besides, the position and order of capture zones for multiplexing were optimised. Both line-based and dot-based layouts were evaluated, with particular attention paid to signal interference, cross-reactivity, and sample depletion effects along the flow direction. The spacing and ordering of capture sites were adjusted to ensure uniform signal development across targets while maintaining a compact strip length (Bartosh, Sotnikov, Hendrickson, *et al.* 2020).

2.5 Techniques for PTC-based heating element

This section describes the development and evaluation of a PTC-based heating element designed for paper-based diagnostic applications. The heating element provides a compact, self-regulating heat source suitable for isothermal amplification, enabling stable temperature control without complex electronics. Its design emphasises compatibility with low-cost fabrication methods, flexible substrates, and battery-powered operation, which are essential for point-of-care use.

The section starts by introducing the materials and equipment used, followed by a discussion of the key electrical and thermal properties of the PTC ink that reveal the heater's self-regulating property. The fabrication process of the PTC-based heating element is then detailed, including the printing and curing steps used to construct the device. The heating performance is subsequently evaluated through electrical and thermal characterisation experiments. Finally, design and process optimisation strategies are presented to improve heating efficiency and integration with the paper-based diagnostic platform.

2.5.1 Material and Equipment

MicromaxTM PTC085 carbon-based PTC resistor was bought from DuPont (DuPont de Nemours, Inc., Wilmington, DE, USA). Silhouette Portrait[®] 2 was bought from Silhouette America (Silhouette America Inc., Lindon, UT, USA). Conductive Silver Paint was purchased from Agar Scientific (Agar Scientific Ltd., Rotherham, England). PicoLog TC-08 high-resolution thermocouple data logger was bought from Pico Technology (Pico Technology Ltd., St Neots, UK). 3.7V Lithium-Ion batteries were bought from RS Components (Corby, UK).

2.5.2 Properties of PTC Ink

Positive Temperature Coefficient (PTC) ink is a composite conductive material characterised by a steep resistivity increase near its switching temperature, which decreases the current of the circuit and prevents the temperature from continuing to rise. It typically consists of a semi-crystalline polymer matrix dispersed with conductive fillers, which can yield a nonlinear rise in resistance. This self-regulating behaviour enables precise thermal limiting and stable heat management in printed electronic and sensing applications (K. Liu *et al.* 2023). In this project, our target temperature is near 65 °C for LAMP amplification.

The physicochemical properties of the PTC ink (MicromaxTM PTC085) used in this project are summarised in Table 2.5, based on the manufacturer's technical specifications (Celanese Corporation 2025). The R magnification factor quantifies self-regulation, showing an increase in resistance as temperature rises. Additionally, due to the high intrinsic sheet resistance (15 – 25 k Ω /sq) and viscosity of the paste, the manufacturer recommends combining the PTC ink with highly conductive silver or carbon inks. This hybrid approach is necessary to minimise contact resistance and produce efficient low-resistance heating elements.

Table 2.5: **Property of PTC ink.**

Properties	Value
Sheet Resistance (k Ω /sq)	15-25
R Magnification Factor (25-85 °C)	8-10
Viscosity (Pa·s)	15-50

2.5.3 Design of the PTC Heating Element

In flexible printed heater fabrication, the choice of substrate, electrode geometry, and deposition method has a significant impact on device performance (Nemomssa *et al.* 2024). Paper substrates offer several advantages over conventional plastic films for disposable printed electronics. Owing to their porous cellulose microstructure, paper enables effective ink wetting, rapid solvent evaporation, and compatibility with low-cost printing processes. Paper is also inexpensive, lightweight, mechanically flexible, and environmentally favourable, making it attractive for disposable diagnostic devices and hybrid electronic–microfluidic systems (Jansson *et al.* 2022, Khan *et al.* 2020).

The PTC heating element used in this thesis was based on the architecture recommended by the manufacturer of the PTC ink (Celanese Corporation 2025). The heater comprised two functional layers: a patterned conductive silver layer and an overlying PTC-ink layer. In this work, the conductive silver layer was formed using conductive silver paint, which functioned as a low-resistance current-distribution layer. Its role was to form the interdigitated electrodes and busbars, distribute current across the PTC coating, and reduce the effective current path through the high-sheet-resistance PTC material. The geometric patterns of the silver IDE array and the assembled heating element are shown in Figure 2.6. The IDE structure consisted of two electrically isolated electrode tracks arranged as interlocking fingers. The PTC ink was applied over the interdigitated region so that the material bridged the alternating silver fingers. This geometry divided the PTC layer into multiple parallel conductive pathways, reducing the effective resistance of the PTC ink and improving its suitability for low-voltage Joule heating.

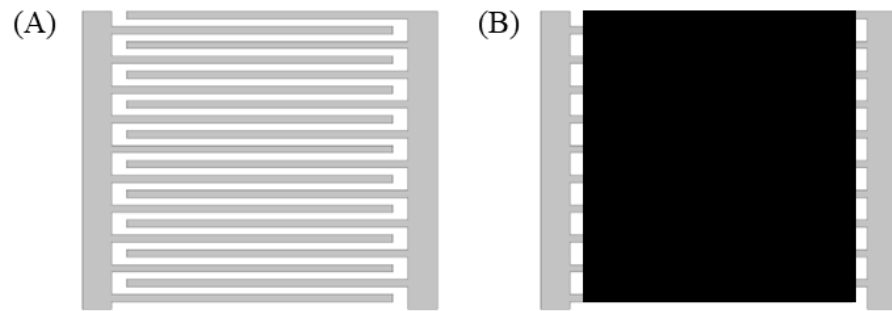


Figure 2.6: **Patterns of the silver circuit and the heating element.** (A) Silver interdigitated electrode (IDE) array. (B) The whole heating element after applying PTC ink.

2.5.4 Prototype Screening

Before the final fabrication protocol was established, a series of prototype-screening experiments was carried out to identify a practical fabrication route and substrate for the PTC-based heater. The screening compared manual deposition, screen printing, and stencil-assisted deposition, as well as discrete and continuous PTC-ink layouts on different substrate materials. The purpose of this stage was to determine which approach provided a suitable balance between pattern definition, electrical resistance, thermal output, reproducibility, and compatibility with the paper-based diagnostic platform.

The first prototypes were fabricated manually to assess the feasibility of the interdigitated silver/PTC heater concept. The paper substrate was pre-printed with a dark background to improve visual contrast during electrode deposition. Silver interdigitated electrodes were then drawn directly onto the substrate using conductive silver ink. Two PTC-ink layouts were tested. In the discrete layout, a laser-cut adhesive film was used as a stencil mask, and PTC ink was applied through the mask to form separated conductive regions bridging the electrode fingers. In the continuous layout, PTC ink was spread across the full interdigitated electrode area to form a single active heating layer.

The best-performing manual prototype was also tested with the PMMA reaction chamber used for liquid-heating measurements. A 30 μL water sample was loaded into the chamber and sealed with a thin film. A thermocouple was inserted through the seal into the liquid to monitor the sample temperature. The heater was attached to the underside of the chamber using double-sided thermally conductive tape, and the liquid temperature was recorded under stepwise voltage inputs of 4, 5, and 6 V.

Screen printing was then assessed as a more scalable manufacturing route. A custom screen mesh was prepared with the inverse pattern of the interdigitated electrodes and PTC regions. Conductive silver paint and PTC ink were applied through the mesh using manual squeegee pressure. This trial was used to evaluate whether the selected inks were compatible with mesh-based deposition and whether the process could provide sufficient pattern resolution for the intended IDE geometry.

Following the screen-printing trial, a stencil-assisted cut-and-print method was developed using the Silhouette Portrait® 2 digital cutter. Disposable stencil masks were cut from 240 μm PVC film and used to define both the silver IDE pattern and the PTC active region. This method was then used for substrate and layout comparison because it provided a low-cost and accessible route for producing repeated prototype patterns without requiring photolithography or reusable mesh screens.

Substrate screening was performed by fabricating discrete-layout PTC heaters on PVC film and cellulose paper. The prototypes were compared in terms of visual integrity after curing, ink spreading, dimensional stability, initial electrical resistance, and thermal response under applied voltage. After the substrate comparison, discrete and continuous PTC-ink layouts were compared on cellulose paper to determine the architecture most suitable for subsequent heater optimisation. The experimental outcomes of this screening process are presented in Chapter 3.

2.5.5 Fabrication of the PTC-Based Heating Element

Based on the prototype-screening results, the final PTC heater was fabricated using a cellulose-paper substrate, silver interdigitated electrodes, and a continuous PTC-ink layer covering the IDE region. The fabrication process is summarised in Figure 2.7.

The fabrication process began with the preparation of a patterned stencil mask for the silver conductive circuit. The circuit layout was designed in Silhouette Studio to define the electrode-finger geometry and the required feature dimensions. The digital pattern was transferred to a Silhouette Portrait® 2 cutter, which produced a disposable stencil mask. The mask was laminated onto the paper substrate so that only the regions corresponding to the conductive traces were exposed. Conductive silver paint was applied over the mask using a paintbrush to form the low-resistance IDE tracks and busbar contacts. After deposition, the mask was removed, and the printed silver circuit was cured on an 80 °C hot plate for 30 s to promote solvent evaporation and adhesion to the paper fibres.

After formation of the silver conductive layer, the PTC ink was applied to form the active heating region. The PTC ink was deposited directly over the cured silver IDEs to create a rectangular active block covering the interdigitated area. Because the PTC layer was deposited manually, the film thickness was not independently controlled. The fabrication process therefore

aimed to produce a visually continuous and sufficiently uniform coating across the active area, while avoiding discontinuous conductive pathways that could increase local resistance variation and thermal non-uniformity. Variation in effective PTC thickness was therefore considered an inherent source of device-to-device resistance variation in this fabrication approach.

After PTC-ink deposition, the complete heater assembly was cured on a hot plate at 130 °C for 10 min to stabilise the PTC composite and ensure electrical integration with the underlying silver circuit. This process produced the printed PTC-based heating element used for the subsequent electrical and thermal characterisation experiments.

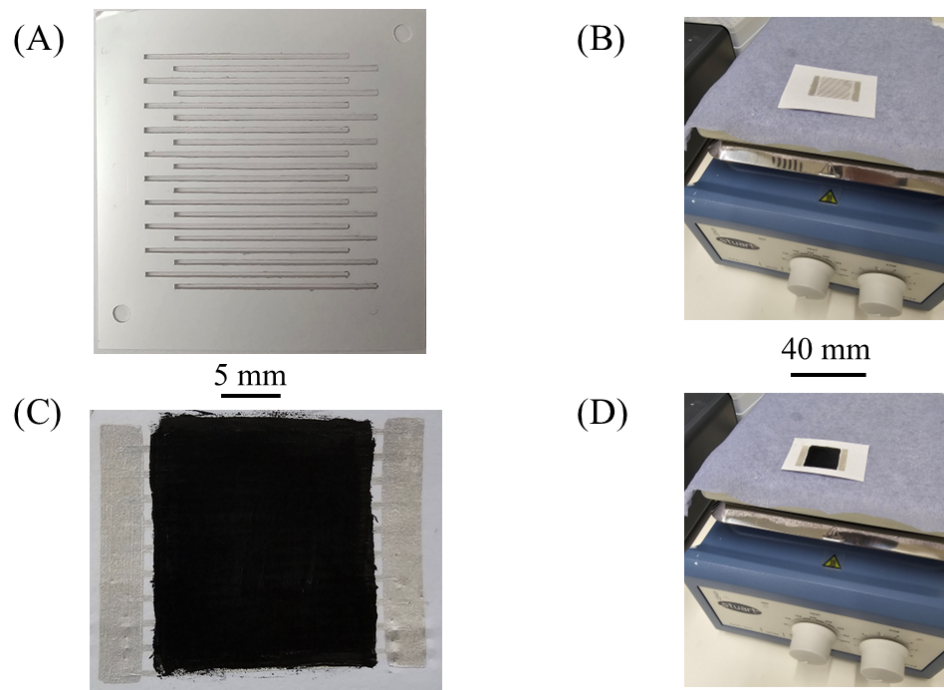


Figure 2.7: **Schematic illustration of the fabrication process for the printed PTC-based heating element.** (A) Design of the conductive circuit layout in Silhouette Studio and cutting of the stencil mask using the Silhouette Portrait® 2. (B) Mask-assisted deposition of silver paint onto the paper substrate, followed by thermal curing at 80 °C for 30 s to form the conductive trace. (C) Application of the PTC ink onto the cured silver circuit to define the rectangular heating block. (D) Final curing of the assembled heating element on a hot plate set to 130 °C for 10 min to complete electrical integration and stabilise the printed layers.

2.5.6 Performance of PTC-based Heating Element

The performance evaluation of the printed PTC-based heating element consisted of two complementary experiments, which are shown in Figure 2.8. First, the electrical resistance of the heating element was measured as a function of temperature. The heating element was placed on a hot plate set to target temperatures between 30 °C and 80 °C in 10-degree increments. At each temperature, after allowing the heating element to equilibrate for two minutes, the resistance was measured using a digital multimeter (set to the Ω function) by contacting the two lead terminals of the printed silver circuit. This procedure ensures that the measured resistance reflects the temperature-dependent behaviour of the PTC-based heating element.

In the second experiment, the heating performance under load was assessed by measuring the temperature evolution of a 30 μ L water sample contained in a small heating chamber in the device. The heating element was powered by a 3.7 V lithium battery, and temperature was recorded using a thermocouple connected to a Pico TC-08 data logger via its corresponding software. The thermocouple measured the water temperature at 1-second intervals over a total period of 30 minutes, providing a detailed temporal profile of heating performance and thermal stability.

These two experiments assessed both the intrinsic electrical behaviour of the PTC-based heating element and its practical heating performance under load. The first experiment characterised how resistance varies with substrate temperature, while the second demonstrates the capacity of the device to raise and maintain the target temperature of a small volume of liquid.

2.5.7 Optimisation of PTC-based Heating Element

The optimisation work focused on two aspects: the geometric configuration of the current printed heating element and improvements in electrode fabrication resolution. For the printed device, two structural parameters were examined: the termination spacing between adjacent IDE fingers and the size of the PTC heating area. Three termination spacings (0.50 mm, 0.75 mm, and 1.00 mm) and six PTC blocks with surface areas of 342, 266, 210, 190, 150, and 110 mm² (corresponding to dimensions of 18 $\times$ 19, 14 $\times$ 19, 14 $\times$ 15, 10 $\times$ 19, 10 $\times$ 15, and 10 $\times$ 11 mm, respectively) were fabricated to assess how electrode spacing and active heating area influence heating efficiency.

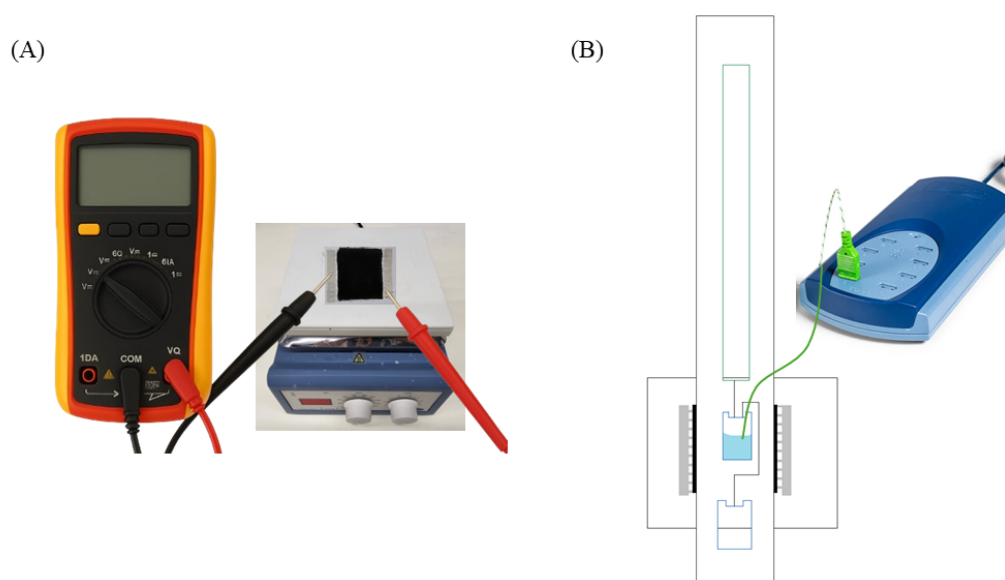


Figure 2.8: **Schematic illustration of the two performance evaluations of the printed PTC-based heating element.** (A) Resistance measurement under defined temperatures (30–80 °C) using a hot plate and multimeter. (B) Heating performance assessment by recording the temperature of a 30 μ L water sample with a thermocouple (TC-08) during battery-powered operation.

Each configuration was evaluated using the previously described water-heating test with a 30 μ L sample in the integrated heating chamber (Figure 2.8). Temperature evolution was recorded over time, allowing comparison of heating rate and maximum temperature among the different geometries. These results were used to identify the most effective balance between heating performance and overall heating element configuration.

To further explore performance limits beyond the resolution achievable by manual printing, micro-scale IDEs were produced through collaboration with Jeff Kettle's group, which is capable of fabricating finer electrode gaps. Devices with 50 μ m and 100 μ m IDE spacing were obtained, enabling investigation of whether denser electrode patterns improve the thermal response of the PTC layer.

The heating behaviour of these micro-fabricated devices was characterised using IR thermography to obtain spatial temperature profiles and evaluate heating uniformity. These optimisations provide a way to improve the efficiency and compactness of the PTC heating element.

2.5.8 Thermal and Electrical Characterisation

The surface temperature distribution and thermal uniformity of the stencil-printed heaters were evaluated using a high-resolution handheld thermal imaging camera (FLIR E40, FLIR Systems, USA). To minimise heat loss via conduction, the heaters were suspended on a thermally insulating foam stage during measurement. A constant DC voltage of 3.7 V was applied to the busbars using a regulated DC power supply. Thermal images were captured once the device reached a steady-state equilibrium (approximately 120 s after activation). Post-process analysis was conducted using FLIR Tools + software. To quantify thermal uniformity, a Line Profile analysis was performed across the horizontal and vertical axes of the active heating area.

To assess the robustness of the heaters for point-of-care applications, three distinct stability protocols were established:

1. **Long-Term Continuous Heating:** The device was powered at 3.7 V continuously for a duration of 60 minutes, simulating an extended LAMP reaction. The temperature of the reaction chamber was logged at 1-second intervals to identify any thermal drift or fluctuation over time.
2. **Cyclic Heating Test:** To evaluate mechanical integrity under repeated thermal expansion, the heater was subjected to 10 consecutive ON/OFF cycles. Each cycle consisted of powering the device for 10 minutes followed by a 10-minute cooling period to return to room temperature.
3. **Shelf-Life Ageing Study:** To verify chemical stability, a batch of heaters was stored under ambient laboratory conditions (25 ± 2 °C, 40%–60% RH) for a period of 9 months. The heating performance (ramp rate and equilibrium temperature) of these “aged” devices was then measured and compared against “freshly” fabricated devices under identical voltage conditions.

The power consumption profile was characterised by monitoring the real-time current draw during operation. A digital multimeter was connected in series with the heater and the 3.7 V power source. The instantaneous current (I) was recorded from the moment of activation ($t = 0$) through the stabilisation phase.

The total energy consumption (E) for a standard amplification reaction was calculated by integrating the instantaneous power ($P = V \times I$) over the reaction duration ($t = 40$ min). Based on the calculated energy demand, the theoretical battery life was estimated assuming a standard single-cell lithium-ion battery capacity of 2600 mAh (9.62 Wh).

2.6 Application of Wax Gate

This section describes the application of a thermally actuated wax gate as a passive flow-control element in the paper-based diagnostic platform. The wax gate is introduced as a temperature-responsive valve that enables timed and automated fluid transfer without the need for external pumps or mechanical components. By utilising the phase-change behaviour of wax, liquid flow can be effectively blocked or released in response to controlled heating, making it well-suited for integration with isothermal amplification and point-of-care diagnostics.

The section first outlines the materials and equipment used for implementing the wax gate, followed by an explanation of its working principle. The fabrication methods are then described, including both printing-based and manual approaches, highlighting the flexibility in wax selection and gate design. Finally, the characterisation of the wax gate is presented, evaluating its sealing performance, actuation behaviour, and compatibility with different heating strategies. These parts establish the wax gate as a reliable and adaptable valve for automated fluid control in paper-based diagnostic devices.

2.6.1 Material and Equipment

Xerox® ColorQube® 8570 Colour Printer and Solid Ink Cartridge were bought from Xerox (Wilsonville, OR, USA), candellila wax was bought from BiOrigins (London, UK). The electric wax melter was bought from GreatArt (London, UK).

2.6.2 Working Principle of the Wax Gate

The wax gate functioned as a thermally actuated phase-change barrier that controlled capillary flow in paper-based microfluidic channels. After curing at an elevated temperature, the solid wax penetrated the paper matrix and formed a continuous hydrophobic barrier that blocked capillary-driven liquid transport. The melting temperature of the selected wax defined the thresh-

old at which the barrier changed from a closed state to an open state (Phillips *et al.* 2016, Carrilho *et al.* 2009). Below this temperature, aqueous samples were unable to penetrate the waxed region, allowing upstream reagents such as LAMP amplification products to be retained during incubation.

When heated above its melting point, the wax changed from a solid to a liquid state. Although this phase transition is thermally reversible in the material sense, the paper-based wax gate was not treated as a reversible or resettable valve because molten wax redistributed within the surrounding paper matrix and did not necessarily return to its original barrier geometry after cooling. This redistribution opened sufficient pore space for liquid transport through the previously blocked region, allowing the naturally hydrophilic cellulose fibres to support flow across the gate (Díaz-González *et al.* 2016, H. P. Kim *et al.* 2025). The wax gate was therefore used as a single-actuation, thermally triggered flow-control element for timed release of LAMP amplicons into the downstream detection zone.

2.6.3 Fabrication of the Wax Gate

Two fabrication approaches were employed to generate wax gates on paper substrates. In the first method, a Xerox ColorQube wax printer was used to deposit solid wax ink, composed of paraffin wax and pigment, directly onto Whatman filter paper. The printed sheets were subsequently placed on a hot plate at 100 °C for 30 s to melt the wax and allow it to penetrate through the paper thickness, forming a complete hydrophobic barrier. After curing, the patterned sheets were cut into 4 mm × 20 mm strips using a paper guillotine.

The second method used an electric wax melter to melt and manually dispense wax onto Whatman filter paper. The wax reservoir was heated until fully liquefied, after which a linear wax gate was drawn by hand onto the paper substrate. The hand-drawn candelilla-wax gates generally produced line widths in the range of 2–4 mm. Because the wax was deposited manually, the gate width was not precisely controlled and the fabrication resolution was not quantified. Instead, gates were first drawn and then selected according to the approximate width required for subsequent timing experiments. This method was therefore used as a proof-of-principle fabrication approach for wax-gate timing rather than as a precision patterning process.

Both fabrication approaches produced hydrophobic barriers capable of blocking capillary flow before thermal actuation, while the manually drawn candelilla-wax gates allowed the melting temperature to be adjusted through material selection, and the wax printer provided higher stability and reproducibility.

2.6.4 Characterisation of the Wax Gate

The functional performance of the wax gate was assessed through a series of characterisation experiments designed to evaluate both its sealing capability in the closed state and its opening behaviour under controlled heating conditions. To verify that the barrier effectively prevented premature fluid transport, 20 μL of a red dye solution was dispensed upstream of the gate on each test strip. The membranes were monitored for 20 min to ensure that no wicking occurred across the wax barrier, confirming complete hydrophobic penetration and a stable closed state.

Two operational modes were investigated: timer-based opening and actuated opening. In the timer mode, the wax gate was positioned within the isothermal amplification chamber and subjected directly to the thermal environment of the reaction. As the chamber temperature remained elevated for 30–40 min, the wax slowly transitioned toward its melting point. The goal of this approach was for the gate to open precisely at the end of the amplification period, allowing reaction products to enter the downstream detection zone. Achieving this behaviour requires careful tuning of wax formulation, gate width, and local heat distribution, making reproducibility a key challenge.

In the actuated-opening mode, the wax gate remained closed throughout the amplification process, ensuring complete containment of reagents in the upstream chamber. After amplification, the gate was selectively opened by applying a localised thermal stimulus. Three heating strategies were tested (Figure 2.9). (A) Hot-plate actuation: Paper strips were placed on a hot plate set to various temperatures for 2 min; successful opening was confirmed when 20 μL of red dye wicked past the melted barrier. (B) Incubator heating: Strips preloaded with dye and sealed in parafilm (to prevent evaporation) were incubated at 65–70 °C for 20–30 min and inspected for dye penetration. (C) Device-integrated actuation: To replicate in-device conditions, wax-gate strips were sealed between a PMMA test chamber and the PCR acetate film, and the previously developed PTC heating element was used as the heat source. Dye solution transport across the gate was then examined to evaluate compatibility with the actual device architecture.

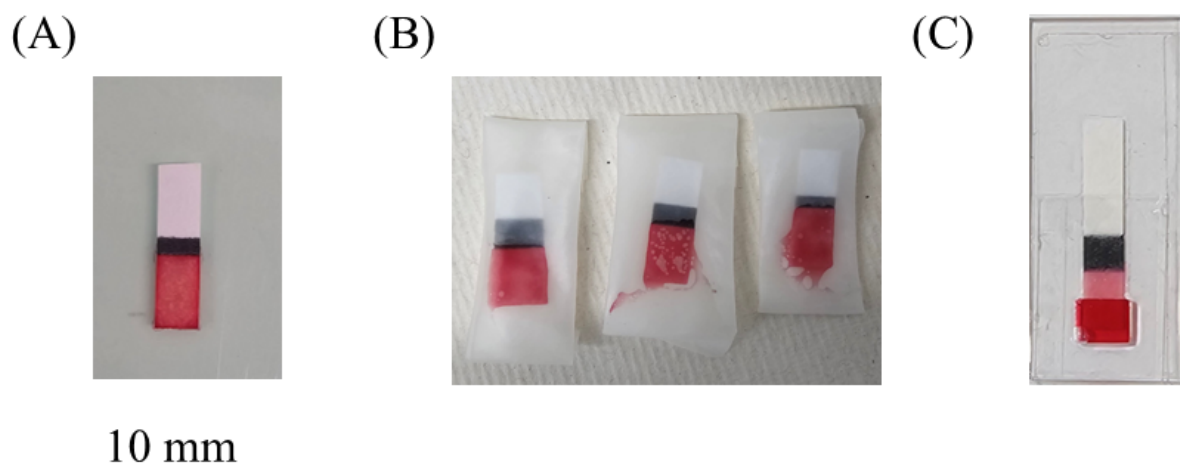


Figure 2.9: **Three actuation strategies used to open the wax gate.** (A) Hot-plate heating: Paper strips were placed on a temperature-controlled hot plate for 2 min to induce melting and gate opening. (B) Dye-loaded strips wrapped in parafilm were heated in an incubator at 65–70 °C and monitored for wax-gate opening over 20–30 min. (C) Device-integrated heating: Wax-gate strips were positioned between a PMMA test chamber and acetate film, and actuated using the PTC-based heating element to evaluate compatibility with in-device thermal conditions.

2.7 Design of Paper-based DNA Amplification and Detection Platform

This section describes the design and development of a paper-based platform for integrated DNA amplification and detection. The aim is to combine isothermal nucleic acid amplification, controlled fluid handling, and lateral flow readout within a compact, low-cost device suitable for point-of-care applications. To achieve this, key functional components (including on-paper LAMP amplification, a PTC-based heating element, and a thermally actuated wax gate) were systematically evaluated and integrated within a PMMA structural frame.

The section first introduces proof-of-concept device architectures used to assess the compatibility of the heating element with existing device designs and to validate the feasibility of wax-gate-mediated flow control in a realistic device environment. These preliminary designs provide essential insights into thermal management, fluid transfer, and user interaction, forming the basis for subsequent optimisation.

Building on these findings, the section then details the design and fabrication of the devices, followed by their operational workflow. Finally, a integrated paper-based platform is presented, in which amplification, flow regulation, and detection are combined into a single device with reduced manual intervention.

2.7.1 Proof-of-concept Device Architecture

Figure 2.10 presents the schematic layout of the paper-based diagnostic devices developed as proof-of-concept platforms for evaluating key components of the integrated system. Two configurations were fabricated: (A) a conventional PMMA-based LAMP-detection device previously used within our group (Guo *et al.* 2021), and (B) a modified device incorporating a thermally actuated wax gate. While both devices share the same base architecture, each is designed to validate different functional aspects before progressing to the integrated platform described later in this chapter.

Device A (Figure 2.10(A)) serves to assess whether the existing PMMA diagnostic structure is compatible with the newly developed PTC heating element. The architecture consists of three principal chambers laser-cut into a PMMA substrate. The reaction chamber, positioned in the middle, accommodates the LAMP amplification mixture. This chamber is typically elliptical or rectangular and sized approximately to a fingertip, providing sufficient volume for both reagent loading and optical/thermal coupling. Immediately below the reaction chamber is the running-buffer chamber, which stores the buffer required to mobilise the amplification product across the detection strip. At the top of the device lies a long, narrow detection chamber containing a standard lateral flow strip. Fluid communication between these parts is established through narrow connecting microchannels, enabling capillary-driven transport once the system is activated. Importantly, the reaction and detection zones are spatially separated because LAMP requires strict thermal isolation, whereas lateral flow detection relies on controlled capillary flow that would be disrupted if exposed to prolonged heating.

Device B (Figure 2.10(B)) was designed to evaluate the performance of the wax gate within a real device environment and to test a simplified workflow where the separate running-buffer chamber is removed. In this compact configuration, the overall geometry is reduced, and a wax valve is incorporated within the channel linking the reaction zone to the detection zone. This thermally responsive barrier maintains separation of the two zones during amplification, preventing premature wicking of the reaction mixture. Upon heating, the wax melts to open the channel and initiate downstream transport, enabling controlled release of the amplicons into the detection strip. This design tests whether valve actuation is reliable under realistic thermal gradients and whether on-chip automation can be achieved without manual loading or pumping of buffer solutions.

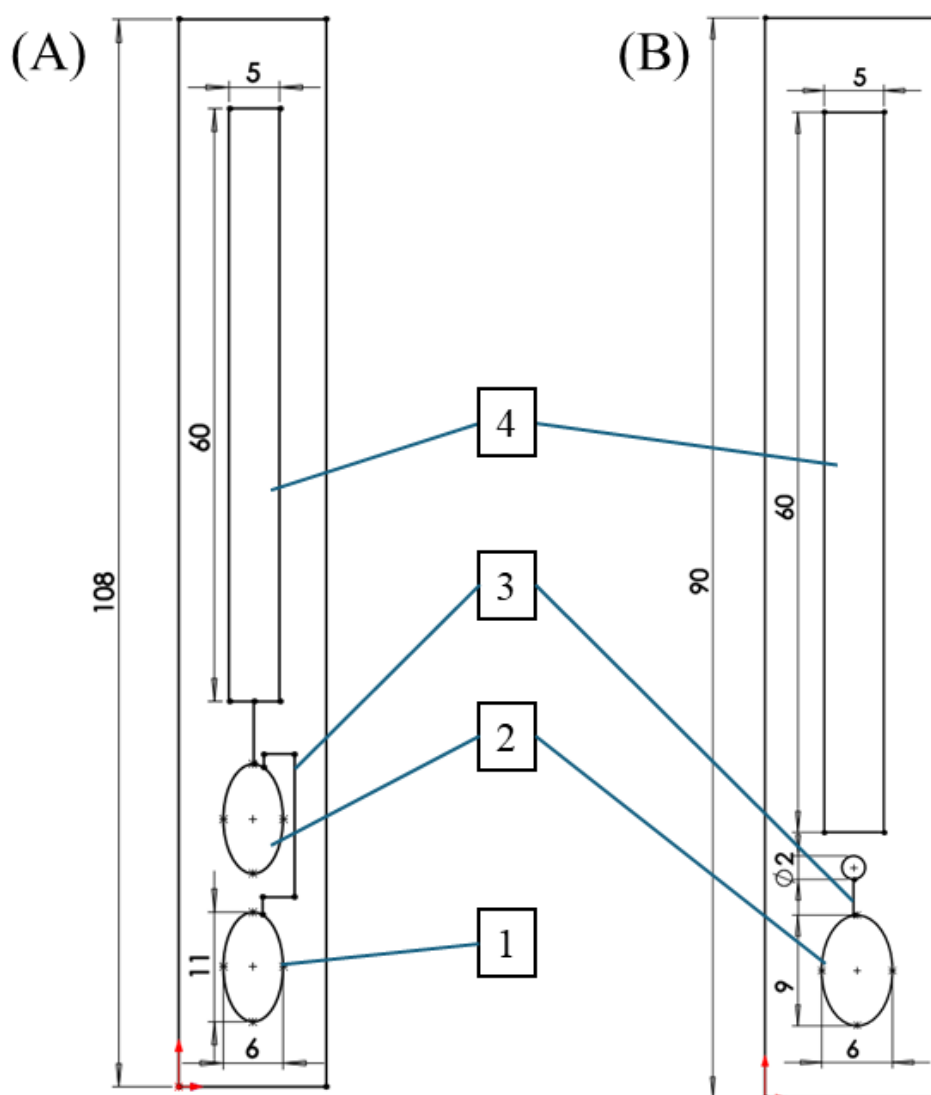


Figure 2.10: **Schematic layout and dimensions of the proof-of-concept PMMA diagnostic devices.** (A) Device A: Standard layout featuring (1) a running-buffer reservoir (11×6 mm), (2) a LAMP reaction chamber (11×6 mm), (3) connecting microchannels, and (4) a detection chamber (60×5 mm) for the lateral flow strip. Total device length is 108 mm. (B) Device B: Wax-gate integrated layout with a compact total length of 90 mm. This configuration removes the separate buffer reservoir and features a smaller reaction chamber (9×6 mm) and a 2 mm contact pore to interface with the wax-gate paper strip.

The heating element, although not shown in the schematic for clarity, is designed as an external and integrable module. A PTC-based self-regulating heater can be adhered to the underside of the PMMA device directly beneath the reaction chamber. Powered by a compact 3.7 V lithium battery, it provides a stable amplification temperature suitable for LAMP while keeping the remainder of the device unheated, thereby preserving the thermal requirements of the lateral flow detection zone. The modular heater arrangement also reduces cost and complexity by allowing the device body (constructed entirely from PMMA) to remain disposable, while the heater can be reused.

PMMA was selected as the structural frame due to its transparency, rigidity, chemical compatibility with LAMP reagents, and excellent suitability for rapid fabrication by laser cutting (Sun *et al.* 2007). These attributes allow accurate definition of microchannels and chamber geometries, improve visualisation during operation, and support a fully enclosed design that enhances biosafety and reduces contamination risk, which is essential for field deployment.

2.7.2 Device Design and Fabrication

The fabrication process can be found in Figure 2.11, which began with the production of PMMA cassettes that form the structural frame of both device configurations. Device layouts were designed using Fusion 360® (Autodesk) or CorelDRAW and imported into RetinaEngrave (Full Spectrum Laser, Las Vegas, NV, USA) for machining. PMMA sheets (1 mm thickness, Stockline Plastic, Glasgow, UK) were laser-cut using calibrated settings (speed 5.00, power 90.0), generating the individual layers and chambers required for assembly.

The specific dimensions of the two proof-of-concept devices are detailed in Figure 2.10. Both devices share a 5 × 60 mm detection chamber to accommodate a standard lateral flow strip, but they differ in the configuration and spatial organisation of the upstream components.

In Device A, the reaction and buffer chambers are both elliptical chambers measuring 6 × 11 mm. These chambers are connected to the detection area via narrow microchannels, each entering from the upper edge of the downstream chamber to prevent backflow and unwanted flooding during capillary-driven transfer. In Device B, the overall layout is made more compact to facilitate integration of the wax gate. The reaction chamber was reduced to 6 × 9 mm, and a small Ø2 mm hole is added at the end of the main connecting channel to ensure complete and reproducible contact between the amplification product and the paper substrate carrying the wax valve. The wax-gate paper strip lies beneath the PMMA layer, bridging the reaction and detection zones while remaining normally closed to prevent premature transport.

Following cutting, all PMMA components were cleaned to remove debris, residues, and potential biological contaminants. Each piece was sequentially immersed in bleach, water, and ethanol under a sterile hood, then left to air-dry on clean tissue. During this time, the acetate sealing films (MicroAmp™ Optical Adhesive Films, Applied Biosystems™, Thermo Fisher Scientific, MA, USA) were trimmed to match the cassette dimensions, and paper strips containing the wax gate (prepared as described in Section 2.6.3) were UV-sterilised for 10 minutes to ensure aseptic handling.

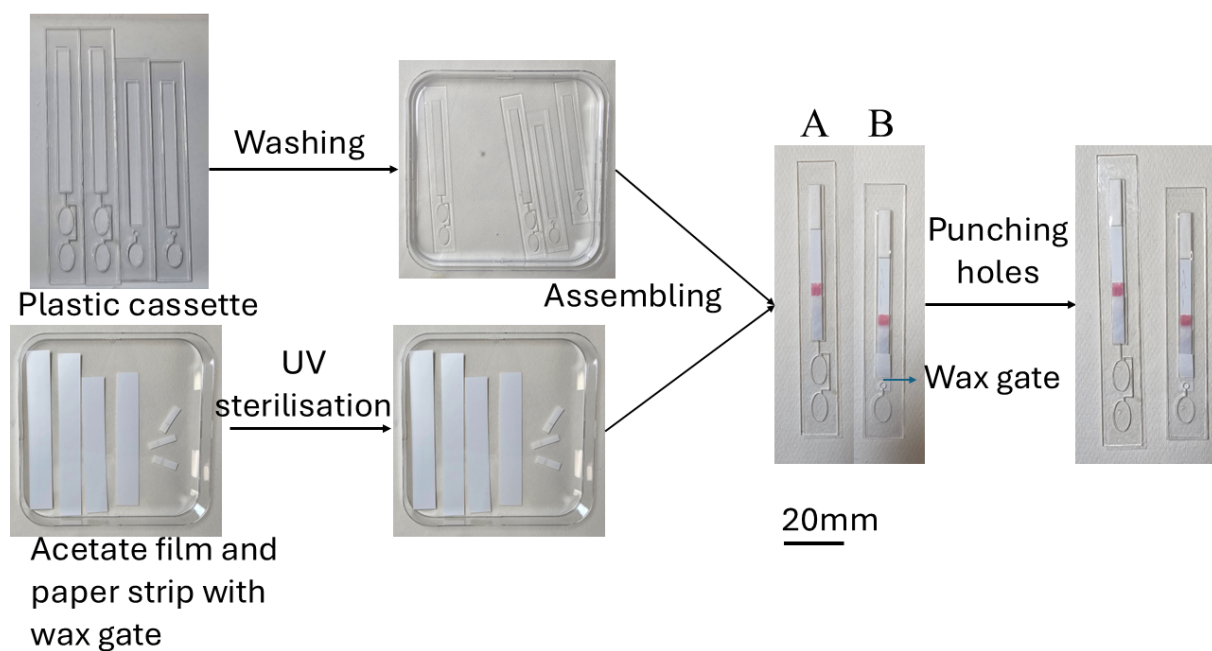


Figure 2.11: **Fabrication procedure for the PMMA diagnostic devices.** Device cassettes were first laser-cut from 1 mm PMMA sheets. Cut components were chemically cleaned and air-dried before assembly. Adhesive acetate films were UV-sterilised and applied to seal the cassette, while wax-gate paper strips were prepared separately and positioned according to the required device configuration. For Device A, the reaction and detection chambers were enclosed with adhesive film and a lateral flow strip was inserted into the detection chamber. For Device B, a wax-gate paper strip was inserted beneath the cassette so that the gate aligned between the reaction and detection chambers, followed by sealing and placement of the lateral flow strip with proper overlap. Finally, $\varnothing 1$ mm inlet holes were punched to permit reagent loading.

Assembly procedures differed slightly between Device A and Device B. For Device A, the underside of the cassette was sealed with acetate film, after which a lateral flow strip was positioned within the detection chamber. A second piece of film was then applied to seal the top surface. Gentle pressure was applied using the blunt end of tweezers to ensure firm adhesion between PMMA and the optical film, creating a fully enclosed device.

For Device B, assembly included integration of the wax-gate paper strip. The strip was inserted beneath the PMMA cassette such that the wax barrier aligned precisely between the reaction chamber outlet and the detection channel. One end of the paper strip overlapped the reaction chamber, while the opposite end extended into the detection chamber. The cassette was then sealed at the back with acetate film. A lateral flow strip was subsequently inserted into the detection chamber, with its sample pad positioned beneath the free end of the paper strip to guarantee proper overlap for capillary transfer. Finally, the upper surface was sealed with a second piece of adhesive film.

The final fabrication step for both devices involved the creation of inlet and buffer-loading holes. A Ø1 mm biopsy punch (Kai Medical, Kai Corporation, Manchester, UK) was used to perforate the designated access points on the sealed chambers, enabling the introduction of reaction mixtures and running buffer during operation.

2.7.3 Operational Workflow

The complete operational procedure of the paper-based diagnostic devices is summarised in Figure 2.12. Devices were prepared and assembled as described in the previous section, after which the assay was conducted through the following sequential steps.

Step 1: Reagent and Sample loading. A 20 µL LAMP reagent mixture (composition listed in Table 2.3, Section 2.1.3) and 5 µL synthetic DNA sample were pipetted into the reaction chamber, followed by the addition of 40 µL running buffer (Section 2.4.2) into the buffer chamber. Once both liquids were loaded, the inlet holes were resealed using fresh adhesive film to prevent evaporation, leakage, or external contamination during the heating process.

Step 2: On-chip amplification. The PTC heating element, powered by a 3.7 V lithium battery, was adhered beneath the device such that the reaction chamber was positioned centrally over the heater's active area. The element was activated for 40 minutes to provide a stable, self-regulated temperature suitable for LAMP amplification.

Step 3: Post-amplification fluid control. For Device A, the heating element was removed after the reaction phase, and the running buffer was manually driven into the reaction chamber via gentle finger-pressing on the buffer chamber. For Device B, the heater was repositioned upward so that the wax gate aligned with the centre of the heating zone. The gate was heated for approximately 1 minute, melting the wax and establishing a fluidic connection between the reaction and detection zones.

Step 4: Transfer to the detection zone. Gentle finger pressure was applied to the reaction chamber, allowing the amplified product to move toward the detection region. In Device A, the liquid travelled through the narrow microchannel into the detection chamber. In Device B, the product wicked along the paper substrate, passed through the now-open wax gate, and entered the lateral flow strip positioned in the detection chamber.

Step 5: Signal development and readout. The lateral flow strip produced test-line signals corresponding to the presence of target amplicons, while the control line confirmed the validity of the strip operation and successful fluid migration.

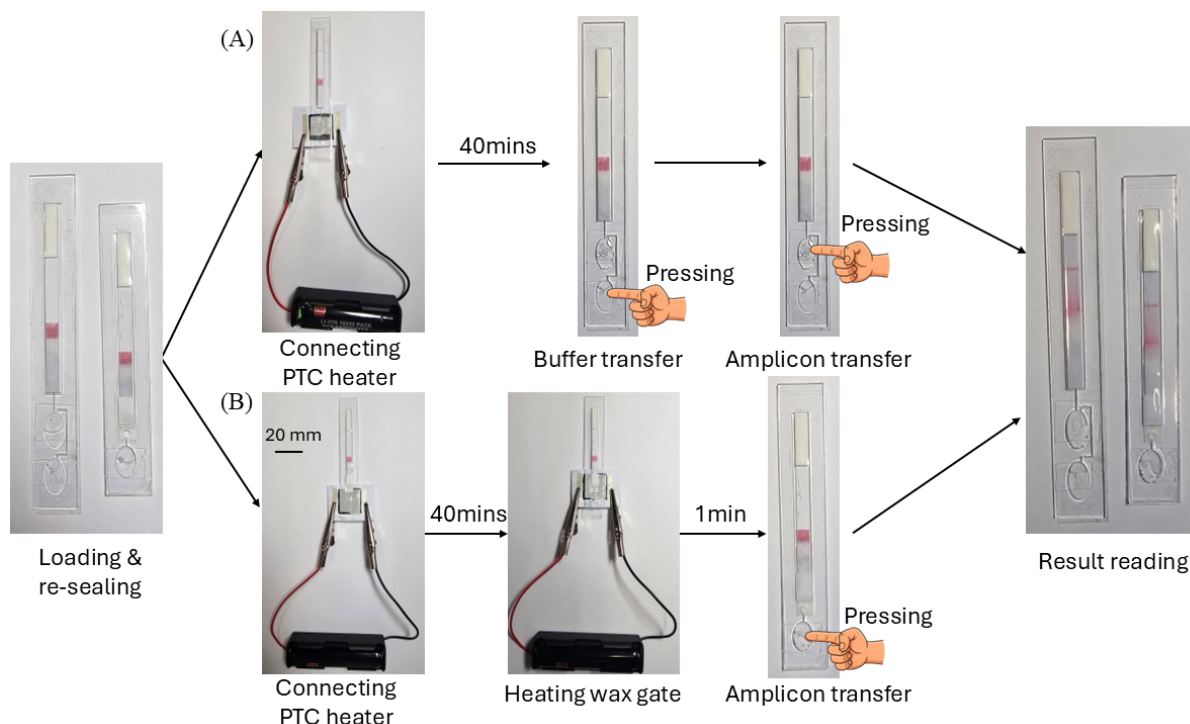


Figure 2.12: **Operational workflow of the integrated LAMP-LFS device.** The device was first assembled and loaded with 25 μL LAMP reagents in the reaction chamber and 40 μL running buffer in the buffer chamber, followed by resealing with an additional adhesive film. The assembled device was then placed on a PTC heating element powered by a 3.7 V lithium battery to perform isothermal amplification. After heating, the running buffer (Device A) or the amplification product (Device B) was directed through the fluidic pathway by gentle finger pressure, enabling transfer to the detection area. For Device B, the wax gate was briefly reheated to open the flow path before product transfer. The resulting LAMP products migrated to the lateral flow strip, where test and control lines provide result readout.

2.7.4 Final Design of the Integrated Paper-based DNA Amplification and Detection Platform

Based on the optimisation results, a final integrated device was developed by combining the PTC-based heating element, thermally actuated wax gate, paper-based LAMP amplification, and lateral flow detection into a single platform. The overall layout, key dimensions, and operational workflow are illustrated in Figure 2.13. This design builds upon the proof-of-concept devices described earlier, integrating the validated components into a simplified and automated diagnostic system.

As shown in the dimensional drawing (Figure 2.13A), the most significant structural modification was the merging of the reaction and detection zones into a single, continuous combined chamber measuring 81×5 mm. This design eliminates the physical PMMA barrier between the two zones, thereby removing the microfluidic channel connecting the reaction chamber and detection chamber. The upstream section of the long rectangular chamber serves as the reaction

zone, where the 50 μL LAMP reaction mixture is loaded directly onto the paper strip. The paper strip bridges the reaction and detection zones, with a wax gate patterned centrally to act as a thermally responsive valve. A separate elliptical buffer chamber ($13 \times 8 \text{ mm}$) is positioned at the base of the device to store the 80 μL of running buffer. This reservoir is connected to the reaction zone via a narrow microchannel designed for controlled buffer transfer. The device has a total length of 113 mm, providing sufficient space for the extended fluidic pathway.

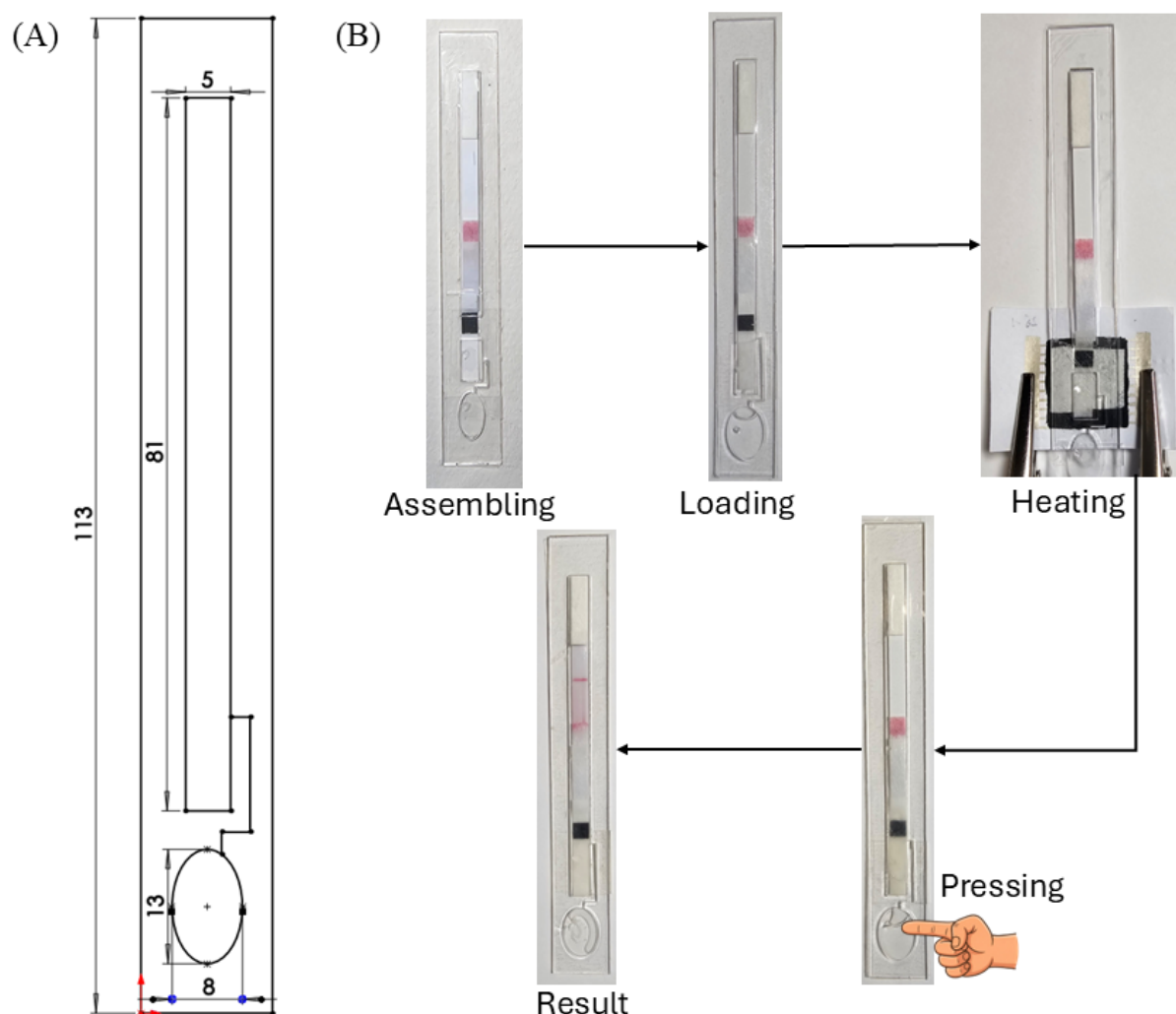


Figure 2.13: **Final design of the integrated paper-based DNA amplification and detection platform.** (A) Dimensional layout of the device showing the rectangular merged reaction and detection chamber ($81 \times 5 \text{ mm}$) and the separate elliptical buffer chamber ($13 \times 8 \text{ mm}$) connected via a microchannel. (B) Operational workflow of the device, illustrating the sequence of reagent loading, on-paper LAMP amplification using the PTC heating element, manual buffer-assisted transfer of amplification products following wax gate actuation, and final lateral flow strip-based result readout.

The internal paper-based components housed within the merged chamber are detailed in Figure 2.14. As shown in the 3D schematic view (Figure 2.14A), a paraffin wax strip was assembled with a lateral flow strip, creating a continuous fluidic path from the upstream amplification area to the downstream absorbent pad. The precise dimensions and overlapping configuration of each functional pad were specified in the cross-sectional diagram (Figure 2.14B). The assembly began with a 24 mm long wax strip, which served as the substrate for the LAMP reaction. A 4 mm wide paraffin wax gate was patterned onto this strip, positioned 14 mm from the upstream edge to isolate the reaction zone. Downstream of the wax gate, a sample pad overlapped the wax strip by 3 mm to ensure robust fluid transfer upon wax gate actuation. The entire assembly extended the total length to 81 mm, fitting precisely within the PMMA chamber.

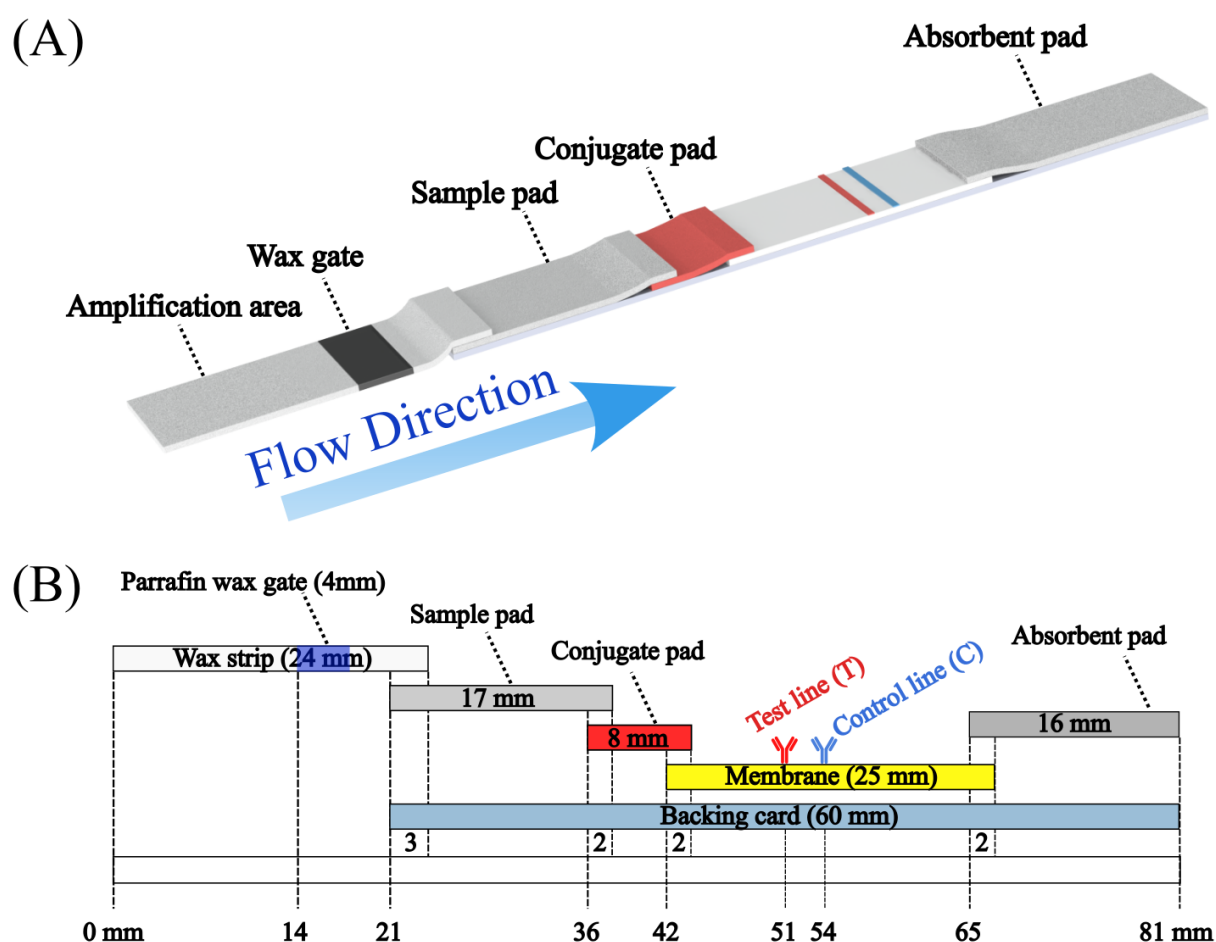


Figure 2.14: **Detailed structure and dimensions of the integrated paper-based lateral flow strip.** (A) 3D schematic view illustrating the sequential arrangement of the functional pads on the backing card, indicating the flow direction from the amplification area (wax strip) through the wax gate to the detection zone. (B) Cross-sectional diagram specifying the precise lengths, positions, and overlap distances of each component: wax strip (24 mm) with a 4 mm wax gate, sample pad (17 mm), conjugate pad (8 mm), nitro-cellulose membrane (25 mm) with Test (T) and Control (C) lines, and absorbent pad (16 mm). The entire assembly was mounted on a 60 mm backing card, creating an 81 mm long continuous fluidic pathway.

The operational workflow (Figure 2.13B) of the final device is streamlined to minimize manual intervention. The lateral flow strip was positioned at the top of the merged chamber. One end of the paper strip overlapped the sample pad of the lateral flow strip by approximately 3–4 mm to ensure reliable capillary contact. Then, several air vents in both chambers were produced using a Biopsy punch after the device was sealed on both sides. 50 μ L reaction mixture was pipetted onto the bottom end of the paper strip, and 80 μ L of running buffer was added to the elliptical buffer reservoir.

For operation, the device was placed onto the PTC heating element, which was powered by a 3.7 V lithium battery. The heating element covered both the reaction area and the wax gate region, allowing simultaneous LAMP amplification and thermal conditioning of the wax barrier over 40 minutes. Following amplification, gentle finger pressure was applied to the buffer chamber to drive the running buffer through the microchannel. This liquid front carried the amplicons through the open wax gate and into the lateral flow strip. The results were then read directly from the test and control lines in the upper detection zone.

By integrating amplification, fluid control, and detection into a single paper-assisted architecture, this final design minimises manual intervention while retaining reliable capillary-driven transport. The combination of a disposable PMMA cassette, modular battery-powered heating, and thermally actuated flow control makes the platform well-suited for point-of-care DNA diagnostics.

Development and Characterisation of a Paper-Based PTC Heating Element for Isothermal Amplification

Effective thermal management is essential for Loop-Mediated Isothermal Amplification (LAMP), which requires a constant temperature of 60–65 °C to ensure enzymatic activity. While this is easily achieved in laboratories, replicating it in portable devices typically requires resistive heaters coupled with complex feedback sensors and control circuits, which increase cost and power consumption (Pardy *et al.* 2017). Common portable heating solutions typically rely on resistive metallic elements coupled with feedback sensors and active control circuits (e.g., PID controllers), which inevitably increase the device cost, form factor, and power consumption.

To address these challenges, this chapter presents the development of a self-regulating heating element based on Positive Temperature Coefficient (PTC) ink printed on paper. By exploiting the material's intrinsic self-regulation at a specific threshold, the heater achieves stable thermal control without the need for active electronics.

This chapter details the design, fabrication, characterisation, and optimisation of the paper-based PTC heating element. The experimental results begin with the material selection and fabrication evolution (Sections 3.1.2–3.1.3). Section 3.1.4 establishes the fundamental electrical behaviour of the heater, specifically characterising the non-linear Resistance-Temperature ($R - T$) relationship that exhibits the self-regulating mechanism. Subsequently, the geometric optimisation of the heating element (including the tuning of electrode gap spacing and heater dimensions to target the 60–70 °C range) is investigated in Sections 3.1.6 to 3.1.7.

Following the design optimisation, the operational performance of the final device is evaluated. This includes an assessment of thermal uniformity via infrared thermography (Section 3.1.8), followed by a comprehensive analysis of operational stability and reusability under continuous and cyclic heating loads (Section 3.1.9). Next, Section 3.1.10 quantifies the power con-

sumption, demonstrating the device's compatibility with low-voltage portable power sources. Finally, the micro-IDEs were investigated for the potential to minimise the heater resistance and power consumption in Section 3.1.11. The results were further discussed in Section 3.2 and 3.3, analysing the physical mechanisms driving the heater's performance and evaluating its scalability and suitability for integration into the final diagnostic platform.

3.1 Results

3.1.1 Theoretical Design Parameters

Before initiating fabrication, analytical design parameters were defined to establish the electrical requirements for the heating element. The primary thermal objective is to maintain a reaction temperature of approximately 65°C using a standard portable power source, such as a 3.7 V lithium-ion battery. Based on thermodynamic principles for microfluidic heating, a power dissipation (P) of approximately 0.5 – 1.0 W is typically required to offset convective heat losses and maintain this temperature in a paper-based device (Kulkarni *et al.* 2024, Pardy *et al.* 2017). Constrained by the requirement for portability, the power source was limited to standard low-voltage batteries (e.g., lithium-ion, coin cells, or AA battery packs), providing a potential difference (V) between 3 and 5 V. Applying Joule's Law ($P = V^2/R$), the target electrical resistance (R) can be derived. Assuming a supply voltage (V) of 5 V and a target power of 1.0 W for rapid heating, the required resistance is calculated as:

$$R = \frac{V^2}{P} = \frac{(5)^2}{1.0} = 25 \, \Omega \quad (3.1)$$

Lower power consumption (e.g., 0.5 W) would permit a resistance up to approximately 50 Ω . Consequently, the fabrication process must target a device resistance in the low-ohmic range of $< 50 \, \Omega$. This theoretical constraint presents a significant challenge: commercial carbon-based PTC inks typically possess high sheet resistance (10 – 25 $k\Omega/sq$). A simple coating of such material would result in resistances in the $k\Omega$ range, generating negligible power ($< 10 \, \text{mW}$). Therefore, to bridge the gap between the material's high intrinsic resistivity and the low target resistance required for battery operation, the conductive ink must be introduced to achieve the low resistance of the heater (Celanese Corporation 2025).

3.1.2 Printing method Screening and Design Evolution

To establish the baseline electrical properties of the commercial PTC ink, characterisations were conducted by depositing the ink onto dielectric substrates. The PTC paste was applied onto PVC films and cellulose filter paper (sample size: 25×10 mm), cured, and subjected to resistance measurements. As shown in Table 3.1, the samples exhibited extremely high initial resistance, ranging from 5 to 38 k Ω , which is consistent with the PTC ink property shown in section 2.5.2. When powered by a desktop DC power supply at 10 V, thermal imaging revealed no significant temperature rise above room temperature (RT). This confirmed that the intrinsic conductivity of the PTC matrix was insufficient to generate the required heating power at low voltages.

Table 3.1: **Initial resistance measurements of PTC ink on PVC film and cellulose paper substrates (four replicates per material).**

Substrate	Test 1 (k Ω)	Test 2 (k Ω)	Test 3 (k Ω)	Test 4 (k Ω)
PVC Film	10	35	38	36
Cellulose Paper	7	5	11	12

In an effort to overcome the prohibitively high intrinsic sheet resistance of the pure PTC ink (> 10 k Ω/sq), a composite layered architecture was investigated. It was hypothesised that introducing a highly conductive silver underlayer would act as a parallel current collector, effectively shunting the high-resistance PTC matrix. According to the parallel resistor model ($1/R_{total} = 1/R_{Ag} + 1/R_{PTC}$), the addition of a low-resistance silver layer ($R_{Ag} \ll R_{PTC}$) should theoretically reduce the bulk resistance of the device by several orders of magnitude, bringing it within the operational range of low-voltage power sources. A base layer of non-PTC conductive silver ink was first printed and cured, followed by one or more top layers of PTC ink. Three different layer combinations were tested, as detailed in Table 3.2. As predicted, this configuration drastically reduced the measured resistance to approximately 50 Ω (a reduction of $> 99\%$). However, despite the improved conductivity, the thermal performance remained inadequate. Under a 10 V bias, the surface temperature failed to rise significantly, likely because the silver underlayer acted as a short-circuit path, bypassing the resistive PTC material entirely and preventing efficient Joule heating.

Table 3.2: **Resistance of layered composite structures comprising conductive silver ink and PTC ink.**

Layer Combination	Measured Resistance (Ω)
1 Layer Conductive Ink + 2 Layers PTC Ink	400
1 Layer Conductive Ink + 1 Layer PTC Ink	150
2 Layers Conductive Ink + 1 Layer PTC Ink	50

Following the limited success of the composite layered approach, the interdigitated electrode (IDE) architecture, suggested by the manufacturer (Celanese Corporation 2025), was investigated. IDEs consist of two interlocking, comb-like metallic arrays separated by a defined gap that force current to flow laterally through the PTC coating.

Analytically, the total resistance (R) of any thin film is defined by the sheet resistance (R_s), the length of the path (L), and the width of the path (W):

$$R = R_s \cdot \frac{L}{W} \quad (3.2)$$

In an IDE, the path length (L) is simply the gap spacing between fingers. The “Width” becomes the total cumulative edge where the positive and negative fingers face each other. This is the Effective Width (W_{eff}). If there are N total fingers, there will be $N - 1$ gaps between them. If each finger has an overlapping length of L_o , the formula is (Kasap 2018):

$$W_{eff} = (N - 1) \times L_o \quad (3.3)$$

For a 20×20 mm heating area with a sheet resistance of $20 \text{ k}\Omega/\text{sq}$, a simple two-terminal block design ($L = 20$ mm, $W = 20$ mm) would yield a resistance of $R = 20 \text{ k}\Omega$. This is too high to generate meaningful heat under low-voltage battery operation ($P = V^2/R = 1.25 \text{ mW}$ at 5 V). In contrast, an IDE architecture places multiple resistive paths in parallel. By reducing the gap (L) to 0.5 mm and utilising 20 interdigitated fingers (effective width $W_{eff} = 19 \text{ gaps} \times 20 \text{ mm} = 380$ mm), the geometric factor (L/W_{eff}) is reduced from 1 to approximately 0.0013. Theoretically, this reduces the total resistance to $R \approx 26 \text{ }\Omega$, enabling a power output of $\approx 0.96 \text{ W}$ at 5 V . This calculation confirms that geometric restructuring via IDEs is not merely an optimisation but a fundamental requirement to utilise high-resistivity PTC inks in low-voltage portable devices. However, this model assumes a uniform and reproducible PTC sheet resistance. In the manually fabricated heaters used in this thesis, this assumption is only approximate because the effective

PTC coating thickness, packing density, and degree of ink penetration into the paper substrate were not independently controlled. Therefore, the IDE geometry was expected to guide the overall resistance trend, but not to fully determine the absolute resistance of each individual heater. To validate this hypothesis, gold interdigitated transducers (IDTs) were acquired from our group member for initial testing (Figure 3.1(A)).

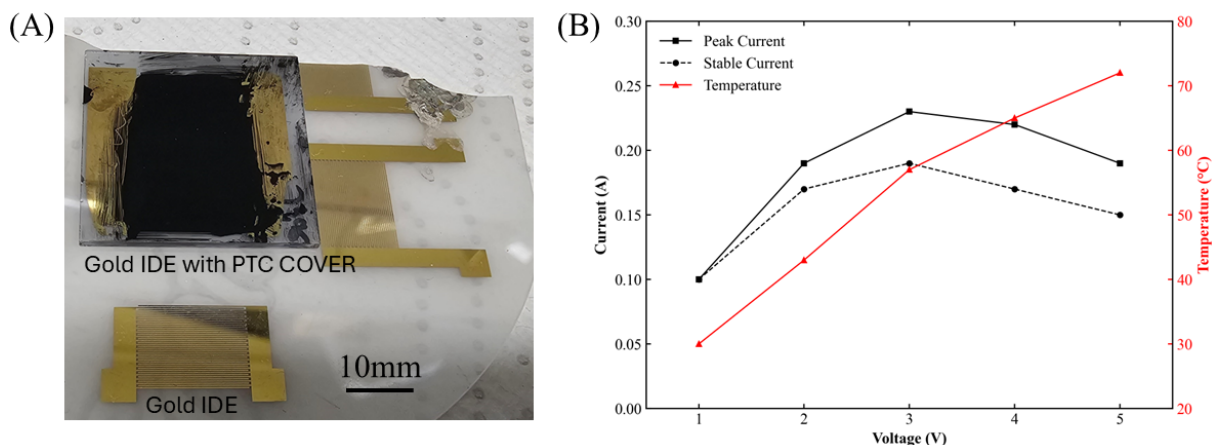


Figure 3.1: **Validation of the interdigitated design concept.** (A) Images of the cleanroom-fabricated gold IDT before and after the application of the PTC ink layer. (B) Electrical and thermal characterisation of the Gold IDT-PTC heater. The plot illustrates the good heating performance and self-regulating effect, where the current (left axis) is dropped as the temperature (right axis) rises.

These standard IDTs are typically fabricated using photolithography and metal deposition processes in a cleanroom environment. Furthermore, the high cost of gold targets and the low throughput of standard lithographic processes render them unsuitable for mass-produced, disposable point-of-care diagnostics (Zamani *et al.* 2023). Despite these economic and manufacturing limitations, they served as an ideal platform to verify the feasibility of the PTC-IDE concept.

A thin layer of PTC ink was brushed onto the gold IDT surface and cured. As shown in Figure 3.1(A), the resistance of the device dropped dramatically to approximately $10\ \Omega$. When powered, the device demonstrated rapid and stable heating performance. The voltage-current-temperature ($V - I - T$) characterisation reveals the characteristic self-regulating behaviour: as the voltage increases from 1 V to 5 V, the current initially rises but is subsequently dropped as the PTC material heats up and its resistance spikes (Figure 3.1B). Notably, at 4 V, the device achieved a stable centre temperature of $65\ ^\circ\text{C}$, perfectly matching the LAMP amplification requirement.

This experiment confirmed that the interdigitated geometry is the key to unlocking the heating potential of high-resistance PTC inks. However, to meet the project’s low-cost requirement, the expensive cleanroom-fabricated gold electrodes had to be replaced with a more scalable alternative. To address this, the fabrication strategy was translated to other easy-to-access printing processes to print conductive silver ink and PTC ink on a paper substrate.

The specific electrode layouts were informed by established designs found in industrial electronics design, where printed PTC elements are widely used for applications such as automotive mirror de-icing (Choi *et al.* 2021). As illustrated in Figure 3.2, two distinct electrode configurations were identified and adapted for this study:

1. **Discrete Block Design (Figure 3.2(A)):** In this configuration, often referred to as the “ladder” pattern, the PTC ink is printed as multiple discrete rectangular blocks that bridge the gaps between the silver electrode fingers. Electrically, this forms a network of resistors in parallel. This design allows for precise tuning of the total resistance by adjusting the number of blocks and minimises ink consumption.
2. **Continuous Coating Design (Figure 3.2(B)):** In this alternative configuration, a single continuous layer of PTC ink is printed over the entire interdigitated area. This design maximises the active heating surface area and simplifies the printing, as the exact positioning of the PTC layer relative to the fingers is less critical than in the discrete block design.

These two industrial-inspired designs formed the basis for the paper-based prototypes. The following section details the fabrication and characterisation of these designs to determine the optimal architecture for the final LAMP heating module.

3.1.3 Prototype Screening Outcomes

Prototype-screening experiments were performed to translate the industrial IDE/PTC concept into a functional paper-based heater suitable for LAMP thermal control. The experimental procedures used for manual prototyping, screen-printing assessment, stencil-assisted fabrication, substrate comparison, and liquid-heating validation are described in Section 2.5.4. This section presents the outcomes of those screening experiments and identifies the heater architecture selected for subsequent optimisation.

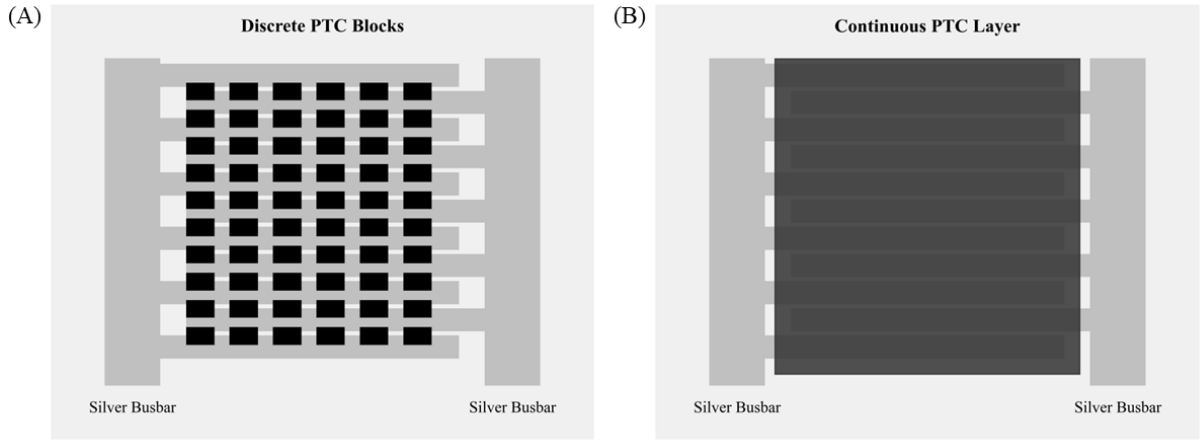


Figure 3.2: **Schematic illustration of the two electrode configurations adapted from industrial standards.** (A) The Discrete Block design, where multiple isolated PTC elements bridge the silver fingers to form a parallel resistor network. (B) The Continuous Coating design, where a single uniform layer of PTC ink covers the entire interdigitated area to maximise surface coverage.

The first screening step compared two manually fabricated PTC-ink layouts: a discrete “Dots” format and a continuous “Fully-covered” format. The discrete layout was originally intended to consist of separated rectangular PTC bridges between neighbouring electrode fingers. However, the thermal nature of laser cutting imposed a constraint on the stencil design. The thin plastic bridges between adjacent apertures deformed during cutting, so the aperture size had to be reduced to preserve mask integrity. As a result, the intended rectangular features became small circular regions resembling dots, as shown in Figure 3.3.

The electrical and thermal performance of the two manual prototypes is summarised in Table 3.3. The “Dots” format showed a high resistance of $600\ \Omega$ and limited heating performance, reaching only $30\ ^\circ\text{C}$ at $5\ \text{V}$. This was attributed to the small volume of PTC ink deposited through the reduced stencil apertures, which was insufficient to form low-resistance conductive pathways across the IDE structure. In contrast, the “Fully-covered” format achieved a much lower resistance of $20\ \Omega$ and generated substantially higher temperatures, reaching $58\ ^\circ\text{C}$ at $5\ \text{V}$ and $80\ ^\circ\text{C}$ at $10\ \text{V}$.

Table 3.3: **Comparison of electrical resistance and thermal performance between the manual “Dots” discrete design and the “Fully-covered” continuous PTC heater design.**

Parameter	Dots (Discrete)	Fully-covered (Continuous)
Resistance (Ω)	600	20
Temperature at $5\ \text{V}$ ($^\circ\text{C}$)	30	58
Temperature at $10\ \text{V}$ ($^\circ\text{C}$)	63	80

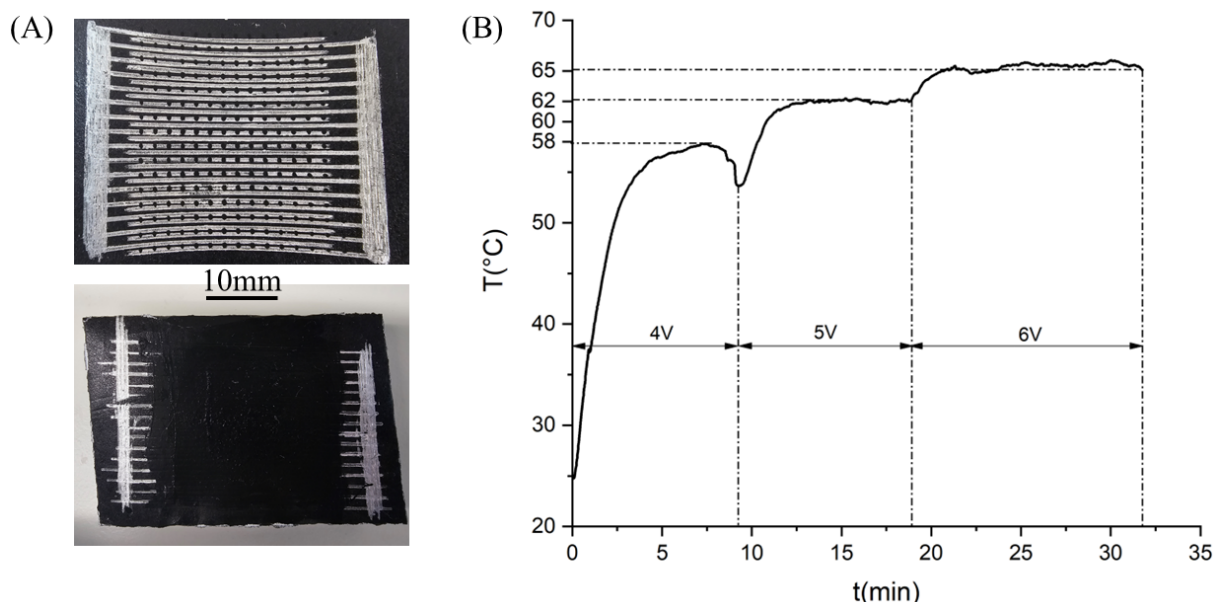


Figure 3.3: **Manual prototype screening and thermal validation.** (A) The images show the hand-drawn discrete “Dots” and continuous “Fully-covered” prototypes. (B) The temperature profile shows the response of a 30 μ L water sample inside the PMMA chamber heated by the manual continuous heater under step voltages of 4, 5, and 6 V.

The continuous manual prototype was then evaluated with the PMMA liquid chamber to determine whether the heater could raise a small liquid volume into the LAMP-compatible temperature range. As shown in Figure 3.3, the liquid temperature increased rapidly after each voltage step and then stabilised. At 6 V, the liquid temperature reached approximately 65.4 °C, confirming that the continuous silver/PTC architecture could provide the required heating range for LAMP.

Although the manual prototype confirmed the feasibility of the heater concept, the fabrication route was not suitable for reproducible device production. Hand-drawn IDEs were time-consuming to produce and showed poor reproducibility. Manual ink deposition also frequently produced short circuits between neighbouring silver fingers because the electrode gaps could not be controlled precisely. These limitations motivated the evaluation of more controlled fabrication routes.

Screen printing was investigated as a potentially scalable alternative because it is widely used in printed electronics. However, the selected conductive silver paint and PTC ink were not compatible with the available screen-printing process. As shown in Figure 3.4, both inks caused severe clogging of the screen mesh. The rapid drying of the silver paint and the high viscosity

of the PTC composite blocked the mesh openings after only a few printing attempts. Cleaning required aggressive solvent treatment and mechanical force, which damaged the screen emulsion. This made the screen-printing route unreliable and unsuitable for the prototyping stage of this project.

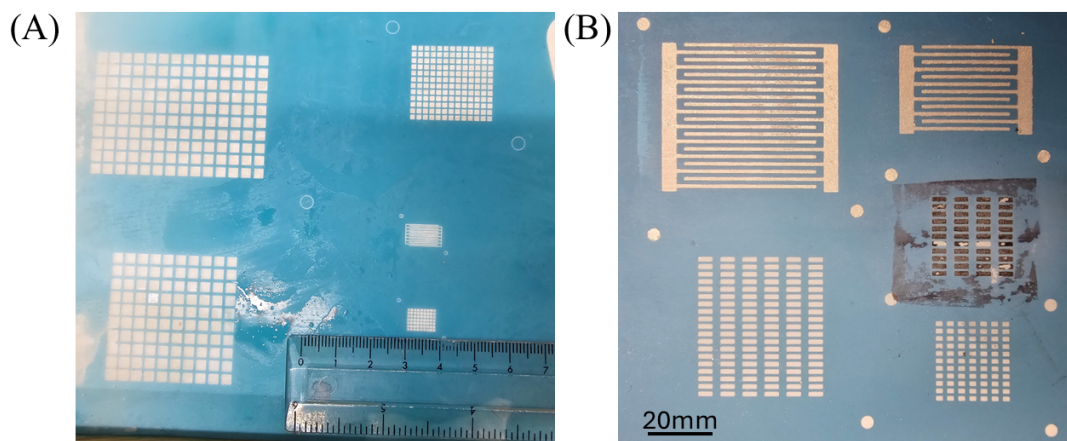


Figure 3.4: **Screen-printing assessment.** The custom screen mesh was designed for electrode and PTC deposition, but the selected silver and PTC inks caused irreversible clogging of the mesh pores and prevented reliable reuse of the screen.

The stencil-assisted cut-and-print route provided a more practical compromise between accessibility, cost, and pattern definition. Disposable masks could be produced rapidly using the desktop cutter, and the method avoided the mesh-clogging issue observed during screen printing. This route was therefore used for the subsequent substrate and layout comparison.

Using the stencil-assisted method, discrete PTC heaters were fabricated on PVC film and cellulose paper. Figure 3.5 compares the printed prototypes and their thermal response under a 5 V DC load. On both substrates, the viscous PTC ink tended to spread beneath the narrow stencil bridges, causing neighbouring printed regions to merge. This indicated that the discrete layout was difficult to reproduce reliably with the selected ink and stencil dimensions.

The two substrates showed different mechanical and thermal behaviour. The PVC-based prototype achieved a lower initial resistance of 19 Ω , but the substrate underwent severe deformation during curing at 130 °C. This warping compromised the structural integrity of the heater and made PVC unsuitable for device integration. The cellulose-paper prototype showed a higher initial resistance of 36 Ω , but it maintained its flatness and dimensional stability during curing and heating. The paper substrate was therefore selected for subsequent heater development because it provided better compatibility with the intended paper-based diagnostic platform.

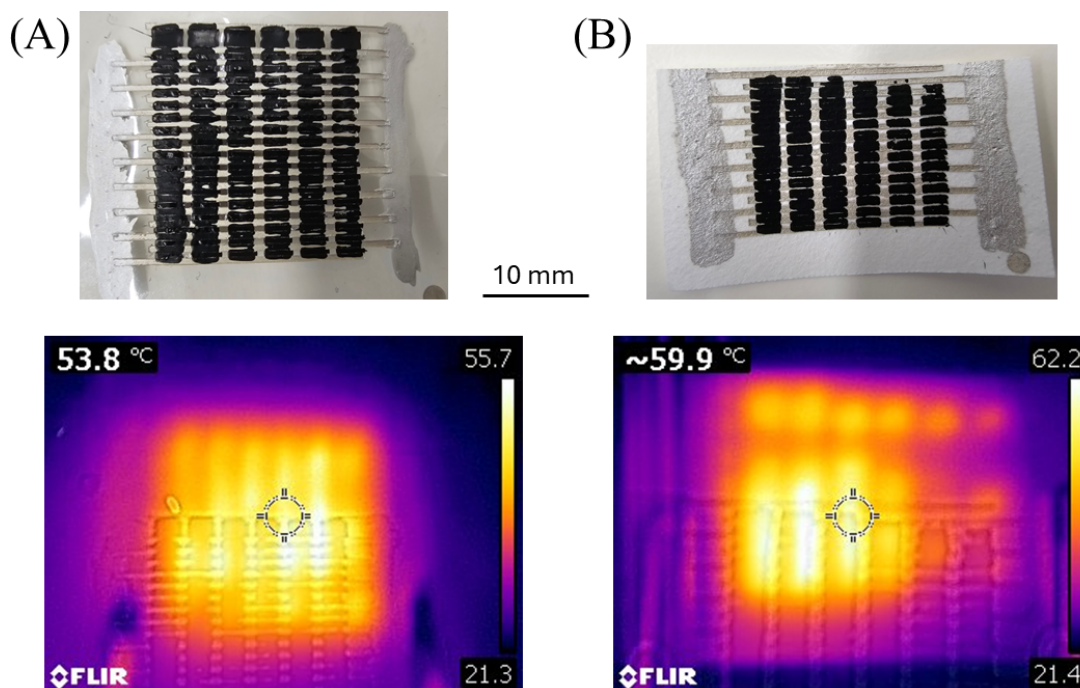


Figure 3.5: **Comparison of discrete PTC layouts printed on PVC film and cellulose paper.** (A) The PVC-film prototype showed lower electrical resistance but deformed during curing. (B) The cellulose-paper prototype showed higher resistance but better dimensional stability and device compatibility. The Ironbow pseudo-colour scale was auto-ranged for each thermal image, with maximum scale limits of 55.7 °C for PVC and 62.2 °C for paper.

After cellulose paper was selected as the substrate, the discrete and continuous PTC layouts were compared on paper, as shown in Figure 3.6. The discrete format remained difficult to control because ink bleeding and merging caused large variation in resistance. Defect-free discrete prints typically showed resistances of approximately 500 Ω , which was too high for efficient low-voltage heating in the intended device configuration. In contrast, the continuous coating produced a substantially lower and more reproducible resistance range of 10–20 Ω and provided a larger effective heating area.

Based on these screening results, the final heater architecture selected for the remainder of the project consisted of cellulose paper as the substrate, silver interdigitated electrodes as the current-distribution layer, and a continuous PTC-ink coating over the active electrode area. This configuration was taken forward for resistance–temperature characterisation, power-source selection, electrode-gap optimisation, active-area optimisation, thermal-uniformity assessment, and liquid-heating evaluation.

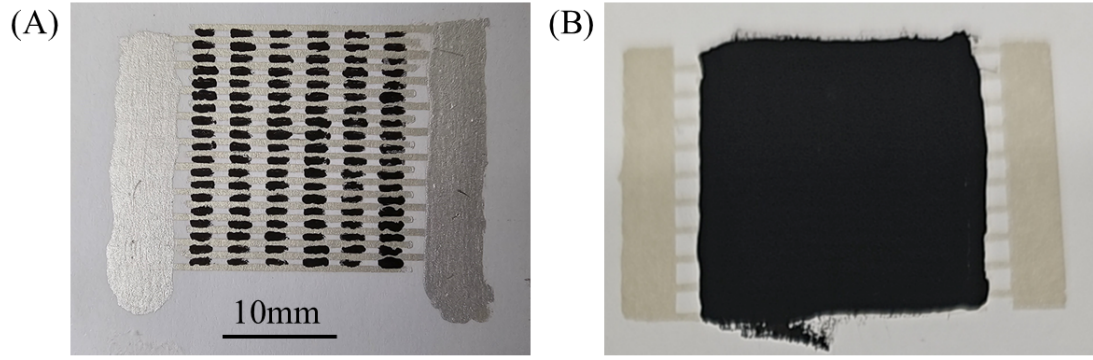


Figure 3.6: **Finalised paper-based PTC heater prototypes fabricated by stencil-assisted deposition.** (A) The discrete-block layout was difficult to reproduce because of ink bleeding and merging. (B) The continuous-coating layout was adopted as the final design because it gave a lower and more consistent resistance range of 10–20 Ω and simplified the fabrication process.

3.1.4 Electrical Characterisation of the PTC Heater

To understand the self-regulating heating mechanism, the resistance-temperature (R-T) characteristics of the fabricated devices were evaluated. As described in Section 2.5.6, the heaters were placed on a hot plate, and their electrical resistance was recorded as the temperature was increased from 30 °C to 80 °C.

Four samples featuring the Continuous Coating design were selected for testing. Due to the inherent variability of the manual stencil printing process, the initial resistance of these samples at room temperature varied between 7.6 Ω and 17.3 Ω . The specific resistance values measured at each temperature step are listed in Table 3.4.

Table 3.4: **Resistance variation (Ω) of four PTC heater samples measured at controlled temperatures on a hot plate.**

Sample	RT	30°C	40°C	50°C	60°C	70°C	80°C
1#	10.2	11.6	16.1	23.6	43.1	85.2	206
2#	7.6	8.2	9.1	11.8	15.8	46.2	121
3#	17.3	18.8	22.7	36.4	56.3	146.7	528
4#	11.1	12.4	17.6	26.9	47.0	129.8	473

The results are plotted in Figure 3.7. All samples exhibited a non-linear Positive Temperature Coefficient (PTC) effect. In the lower temperature range (30–50 °C), the resistance increased gradually. However, as the temperature approached the target range of 60–80 °C, the resistance rose sharply. This rapid increase in resistance is the mechanism that restricts current flow and prevents the device from overheating.

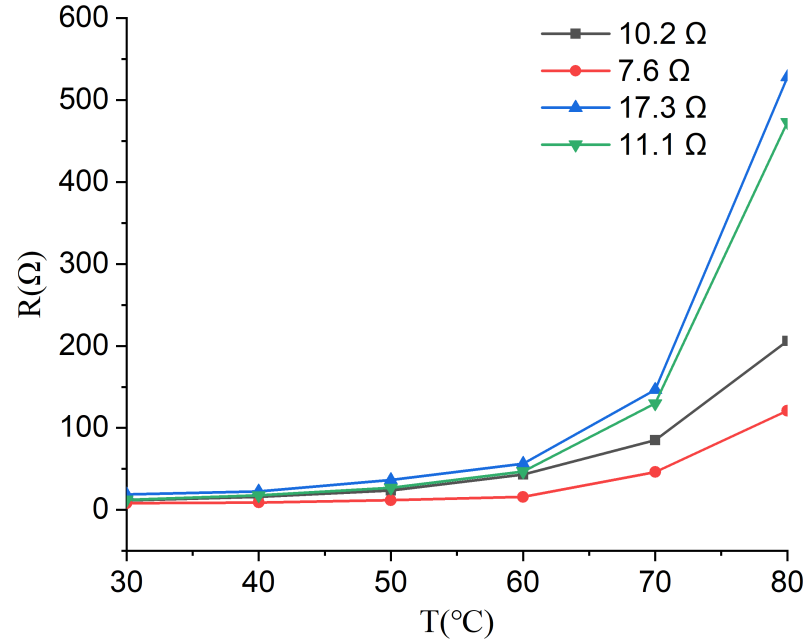


Figure 3.7: **Resistance-Temperature (R-T) characteristics of four paper-based PTC heaters with varying initial resistances.** The graph shows that samples with lower initial resistance exhibit a less drastic rise in resistance at high temperatures, allowing for higher power retention.

The data reveal a correlation between the initial resistance and the rate of resistance change (slope). Samples with a higher initial resistance (e.g., 17.3 Ω) exhibited a much steeper increase at high temperatures compared to samples with lower initial resistance (e.g., 7.6 Ω). For instance, at 80 °C, the resistance of the 7.6 Ω sample rose to 121 Ω, whereas the 17.3 Ω sample spiked to 528 Ω. This behaviour has a direct impact on the heating performance. Since the heating power is defined by $P = V^2/R$, a sharp increase in resistance causes a rapid drop in power. If the resistance rises too quickly (as seen in the high-resistance samples), the power output drops below the level needed to sustain high temperatures. Take sample 3# as an example. As the temperature reached 60 °C, the resistance of this sample spiked to 56.3 Ω. Under a 3 V supply, this resistance resulted

in a power output of only:

$$P = \frac{V^2}{R} = \frac{(3)^2}{56.3} \approx 0.16 \text{ W} \quad (3.4)$$

which is far below the 0.5 – 1 W power we discussed in section 3.1.1. Therefore, to achieve higher equilibrium temperatures suitable for LAMP (65 °C), a heater with lower initial resistance is preferred, as it maintains sufficient power density before the self-limiting effect fully engages.

3.1.5 Power Source Selection

A critical requirement for Point-of-Care Testing (POCT) devices is portability. While a desktop DC power supply offers precise voltage control for laboratory characterisation, it is bulky and requires a mains connection, making it unsuitable for field applications. Initially, button batteries were considered the ideal power source due to their extreme compactness, disposability, and widespread availability. To validate this choice, a comparative study was conducted to evaluate the heating performance of the heaters when driven by different power sources.

Three heater samples (Samples 1#, 2#, and 3#) were tested using four different power sources, as illustrated in Figure 3.8(A): Desktop Power Supply, Lithium-ion Battery, and Button Battery(from top to bottom). The equilibrium temperatures achieved under each power source were recorded and are summarised in Table 3.5 and plotted in Figure 3.8(B).

Table 3.5: **Comparison of equilibrium temperatures (°C) achieved by PTC heaters using different power sources.** Samples 1#, 2#, and 3# possess initial resistances of 8.3 Ω, 9.8 Ω, and 10.8 Ω, respectively.

Sample	Resistance (Ω)	3V Supply	3V Button Batt.	3.7V Supply	3.7V Li-ion Batt.
1#	8.3	60	35	63	66
2#	9.8	55	38	59	64
3#	10.8	54	39	57	62

The results reveal a critical limitation of the button batteries. While the 3 V laboratory power supply successfully heated Sample 1# to 60 °C, the 3 V button battery failed to generate sufficient heat, reaching only 35 °C. This poor performance is attributed to the high internal resistance of standard button batteries. Since the printed PTC heaters have a low resistance (8–11 Ω), a significant portion of the energy was dissipated internally within the battery itself rather than being delivered to the heater.

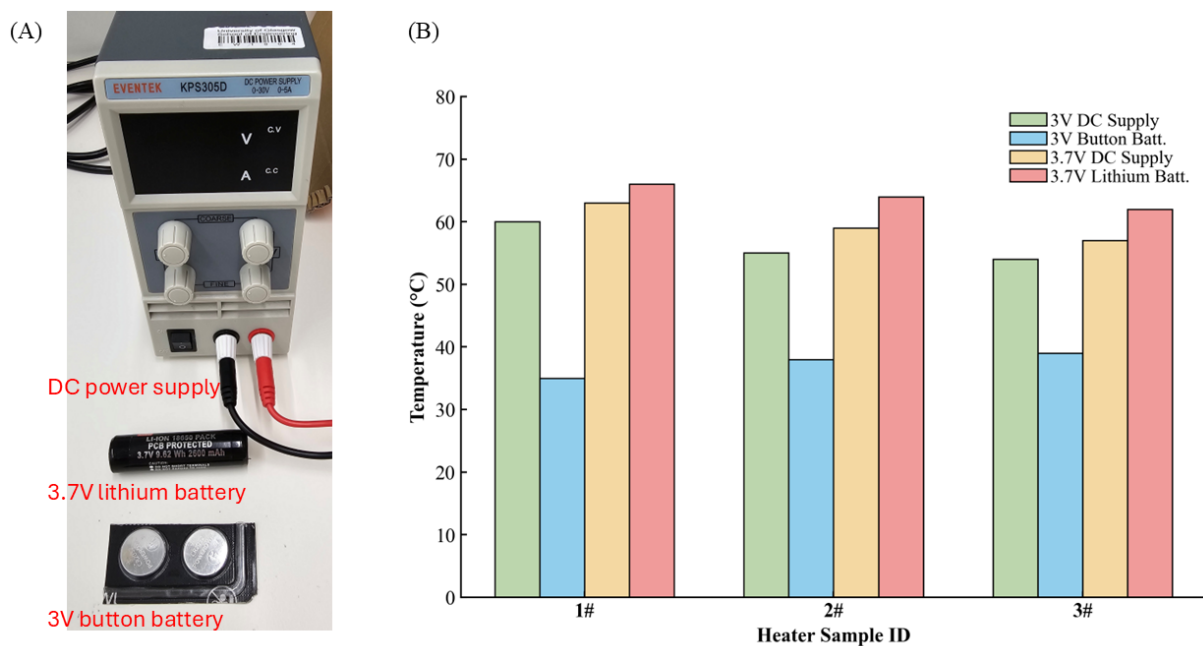


Figure 3.8: **Selection of the optimal power source.** (A) Optical images of the tested power sources: Desktop DC power supply (top), Lithium-ion battery (middle), and Button battery (bottom). (B) Thermal performance comparison of PTC heaters powered by these sources. The Lithium-ion battery successfully drives the heater to the target temperature, whereas the button battery fails due to high internal resistance.

In contrast, the 3.7 V Lithium-ion battery demonstrated excellent performance, matching or even slightly exceeding the temperatures achieved by the 3.7 V desktop supply. All three samples reached the target LAMP temperature range (60–65 °C) when powered by the Lithium-ion battery. This confirms that the Lithium-ion battery possesses the necessary current discharge capability to drive the low-resistance heaters effectively.

Based on these findings, the desktop power supply was retained for controlled laboratory characterisation, while the Lithium-ion battery was selected as the power source for the final portable device.

3.1.6 Optimisation of Electrode Gap Spacing

The geometry of the interdigitated electrodes (IDEs) directly influences the electrical resistance and, consequently, the thermal output of the heater. Specifically, the spacing between the electrode fingers defines the effective length of the resistive PTC material. To determine the optimal geometry for LAMP amplification, three sets of heaters were fabricated with electrode gaps of 0.5 mm, 0.75 mm, and 1.0 mm.

The selection of this specific range was determined by the trade-off between manufacturing constraints and electrothermal efficiency. The 0.5 mm gap represents the practical lower limit defined by the resolution of the mask cutting using Silhouette Portrait® 2 and manual printing process; reducing the spacing further significantly increased the incidence of short-circuit defects due to electrode bridging. Conversely, the 1.0 mm gap was established as the theoretical upper limit. According to Equation 3.2, widening the gap to 1.0 mm theoretically doubles the resistance compared to the 0.5 mm baseline. As discussed in Section 3.1.4, such an increase would degrade the power output to a level insufficient for sustaining the reaction temperature. Consequently, extending the investigation to gaps larger than 1.0 mm was deemed unnecessary.

Five replicates were produced for each gap size to assess reproducibility. The electrical resistance of each sample was measured using a digital multimeter, and the steady-state surface temperature was recorded using an IR camera under a constant 3 V DC supply.

The results are summarised in Figure 3.9. A clear correlation exists between the gap size, electrical resistance, and the resulting temperature. As the gap width increased, the resistance of the heater increased significantly. The 0.5 mm design exhibited the lowest average resistance (approximately 9.5 Ω), while the 1.0 mm design showed the highest resistance (approximately 23 Ω). This trend aligns with theoretical expectations, as a wider gap increases the path length for the current travelling through the resistive ink.

Since the heating power is inversely proportional to resistance, the increase in resistance resulted in a decrease in surface temperature. Under the 3 V supply, the 1.0 mm and 0.75 mm gap designs reached average temperatures of only 45 °C and 51 °C, respectively, which are insufficient for LAMP reactions. In contrast, the 0.5 mm gap design achieved an average temperature of approximately 63 °C. This value falls directly within the optimal range for Bst polymerase activity (60–65 °C). Therefore, the 0.5 mm gap spacing was selected for the final device architecture.

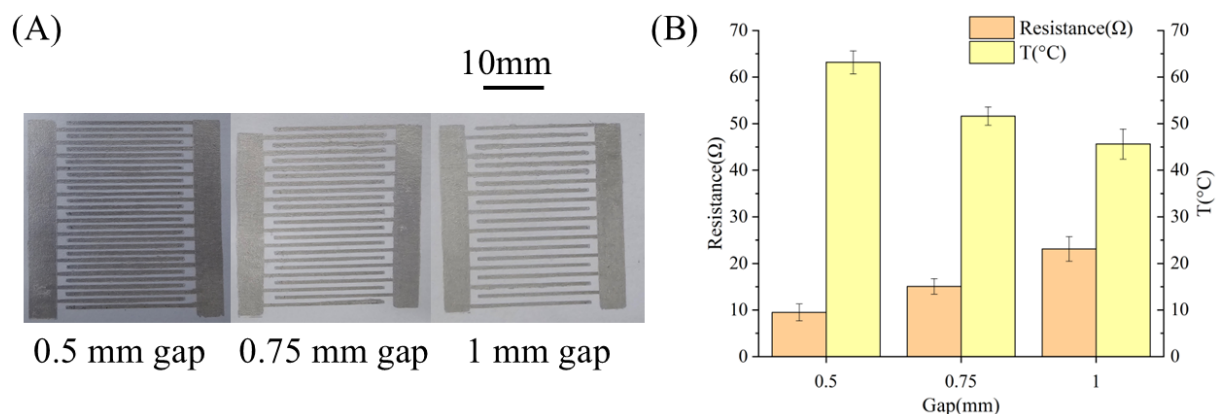


Figure 3.9: **Influence of electrode gap spacing on heater performance.** (A) Optical images of the silver interdigitated electrodes with gap sizes of 0.5 mm, 0.75 mm, and 1.0 mm. (B) Bar chart showing the measured resistance and equilibrium temperature for each gap size under a 3 V supply ($n = 5$). The 0.5 mm gap design yields the lowest resistance and successfully achieves the target LAMP temperature.

3.1.7 Optimisation of Heating Area Size

The overall dimensions of the heating element define the total power generation and determine the spatial uniformity of the thermal field. The physical constraints of the integrated diagnostic cassette primarily governed the selection of these dimensions. The standard width of the PMMA device is approximately 14 mm, with an initial reaction chamber length of approximately 15 mm. To ensure complete thermal coverage of the reaction zone without wasting energy on the surrounding substrate, the baseline heater footprint needed to closely align with these dimensions.

To systematically investigate the influence of heater size on performance, the active heating area was defined by two variables: the overlapping finger length (L_o) and the total array width (W_a). The inter-electrode gap spacing (L) was kept constant at 0.5 mm, and the electrode finger width was also fixed at 0.5 mm. The definitions of these geometrical parameters are shown schematically in Figure 3.10. In this schematic, W_a refers only to the width of the active interdigitated array, measured from the top edge of the uppermost finger to the bottom edge of the lowermost finger, while L_o defines the overlapping finger length within the PTC-coated heating area.

Before fabrication, the theoretical resistance of each geometric configuration was projected using the established ideal thin-film model (Equations 3.2 and 3.3). For the IDE geometry used in this work, the resistance was estimated as:

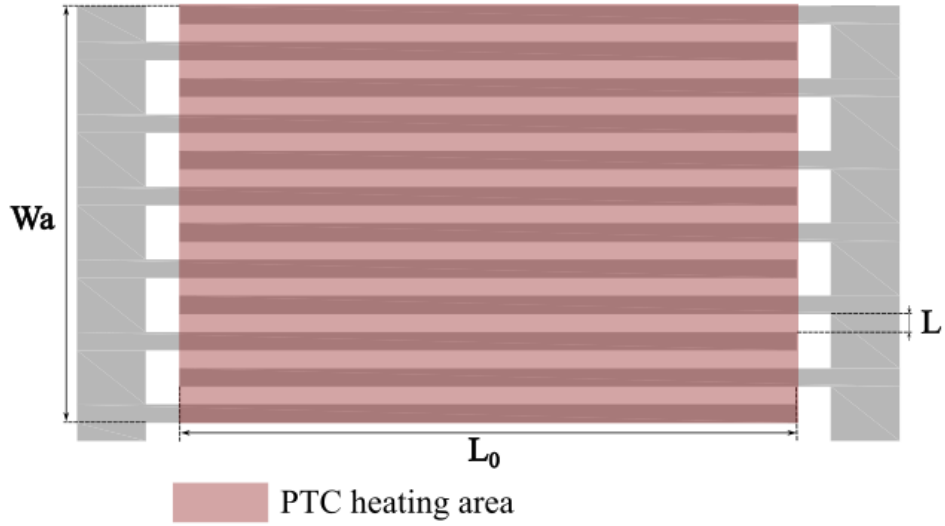


Figure 3.10: **Schematic definition of the IDE geometrical parameters used for resistance estimation.** The active PTC heating area is defined by the overlapping finger length (L_o) and the total array width (W_a). The parameter L denotes the gap spacing between adjacent opposing electrode fingers, while the individual electrode finger width was fixed at 0.5 mm. The grey regions represent the silver interdigitated electrodes, and the shaded region represents the PTC heating area.

$$R = R_s \cdot \frac{L}{(W_a / (0.5 + L) - 1) \times L_o} \quad (3.5)$$

Given a constant gap spacing (L) of 0.5 mm, a fixed electrode finger width of 0.5 mm, and a nominal sheet resistance (R_s) of 20 k Ω /sq, six configurations with varying $L_o \times W_a$ dimensions were calculated: 18 $\times$ 19 mm, 14 $\times$ 19 mm, 14 $\times$ 15 mm, 10 $\times$ 19 mm, 10 $\times$ 15 mm, and 10 $\times$ 11 mm, corresponding to active areas from 342 to 110 mm². The theoretical resistances for these areas were projected to be approximately 30.9 Ω , 39.7 Ω , 51.0 Ω , 55.6 Ω , 74.4 Ω , and 100.0 Ω , respectively.

Based on the power requirements established in Section 3.1.1, resistances above 50 Ω would theoretically fail to generate sufficient heat under a low-voltage portable supply. To evaluate these empirical limits and assess process reproducibility, four replicates of each configuration were produced. The initial electrical resistance of each sample was measured at room temperature using a digital multimeter. The results are compared in Figure 3.11.

The data indicate a clear inverse relationship between the physical size of the heater and its electrical resistance. As shown in Figure 3.11 (A), the largest configuration (18 $\times$ 19 mm) exhibited the lowest average resistance (7.7 Ω), while the smallest configuration (10 $\times$ 11 mm) displayed the highest average resistance (51.0 Ω). This trend aligns with the physical principles of

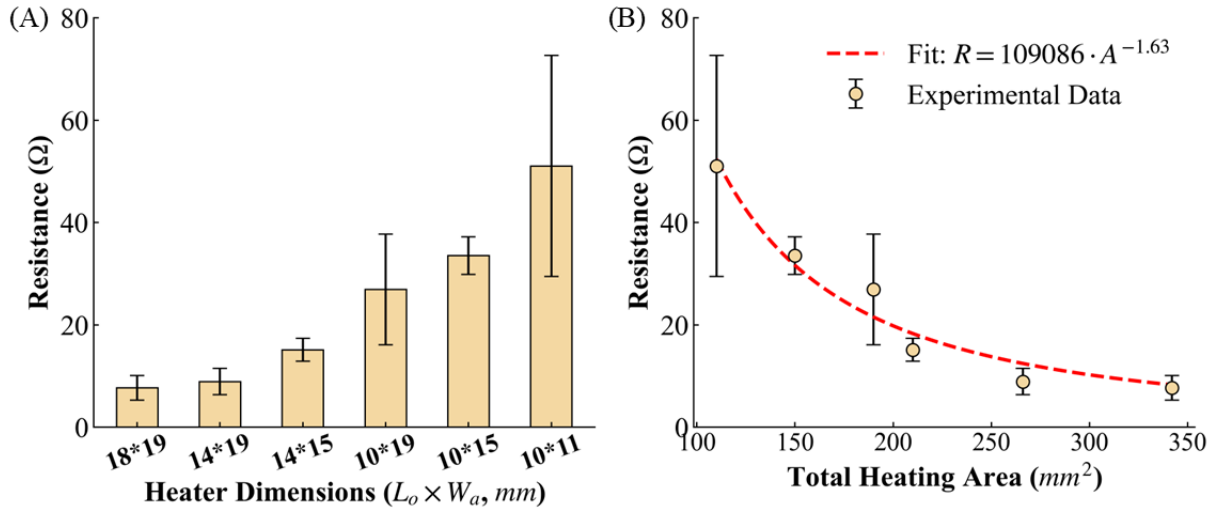


Figure 3.11: **Initial resistance of PTC heaters as a function of active area dimensions.** (A) Average initial resistance for six different heater configurations, defined by overlapping finger length (L_o) and array width (W_a). Error bars represent the standard deviation of four replicates ($n = 4$). (B) Power-law regression demonstrating the non-linear inverse relationship between total heating area and resistance, yielding an exponent of -1.63. A clear trend is observed where resistance increases, and fabrication reproducibility decreases as the heater dimension is reduced.

interdigitated electrodes, which function as a network of resistors in parallel. Increasing the array width (W_a) adds more electrode fingers, creating additional parallel current paths. Similarly, increasing the overlapping finger length (L_o) expands the effective cross-sectional area available for current flow.

To quantify this scaling behaviour, the resistance was plotted against the total heating area (Figure 3.11 (B)). A power-law regression of the experimental data ($R \propto A^{-1.63}$) revealed that the resistance drops more rapidly with increasing area than predicted by an ideal 2D theoretical model (which would yield a perfect hyperbolic exponent of -1). This steeper geometric penalty indicates that maximising the total heating area is disproportionately effective at reducing the overall resistance of the device.

Furthermore, the variation between replicates (indicated by the error bars) increased significantly as the heater dimensions decreased. Large heaters (18×19 mm) demonstrated high reproducibility with a narrow standard deviation. In contrast, the smallest heaters (10×11 mm) exhibited severe inconsistency, with resistance values fluctuating by over 20 Ω between samples. This suggests that miniaturised geometries are highly sensitive to the tolerances of man-

ual stencil printing. In a small active area, minor inconsistencies in ink thickness account for a disproportionately large fraction of the total conductive path, leading to drastic fluctuations in resistance. Conversely, larger heating areas successfully average out these microscopic local defects, resulting in highly stable and predictable electrical properties.

Following the electrical characterisation, the thermal performance of each configuration was evaluated under realistic operating conditions. As detailed in Section 2.5.6, a 30 μL water sample was loaded into the PMMA reaction chamber, and the selected 3.7 V Lithium-ion battery powered the heater. The temperature of the liquid was monitored in real-time using a fine-gauge thermocouple.

Figure 3.12 presents the temperature profiles for the six heater geometries over the first 10 minutes of operation. A direct correlation is evident between the heating area, the electrical resistance, and the resulting equilibrium temperature.

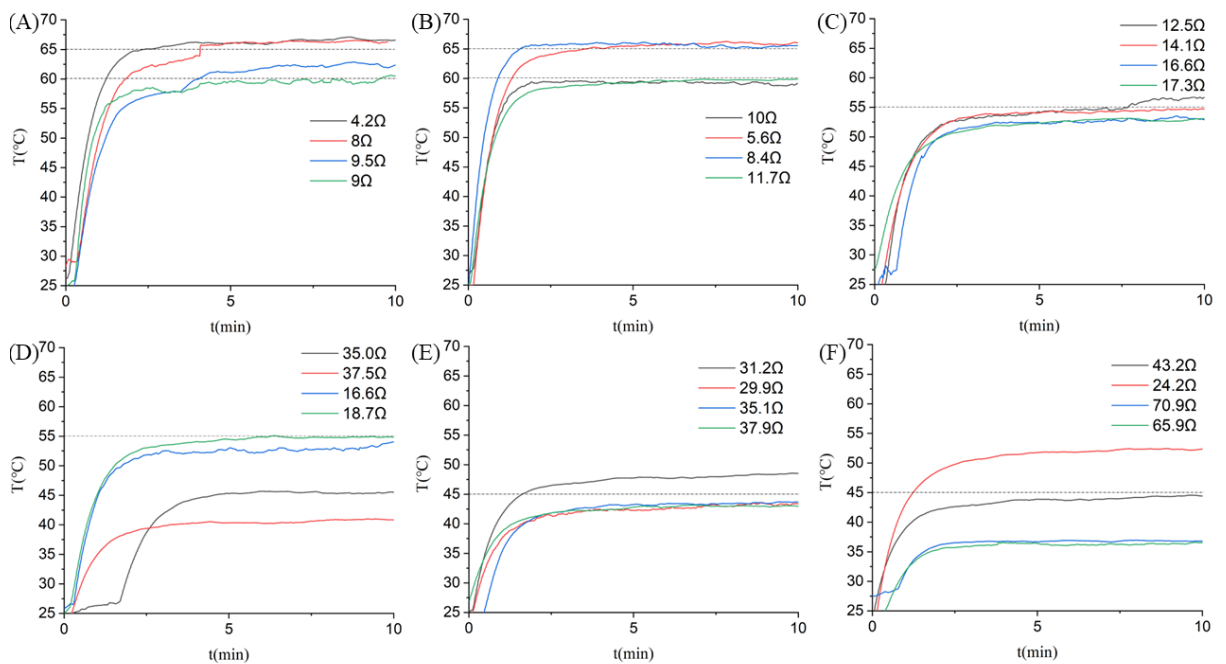


Figure 3.12: **Transient temperature profiles of water samples heated by different heater geometries.** The plots display the temperature evolution of 30 μL water inside the chamber. (A) $18 \times 19 \text{ mm}$, (B) $14 \times 19 \text{ mm}$, (C) $14 \times 15 \text{ mm}$, (D) $10 \times 19 \text{ mm}$, (E) $10 \times 15 \text{ mm}$, and (F) $10 \times 11 \text{ mm}$. Both the $18 \times 19 \text{ mm}$ and $14 \times 19 \text{ mm}$ designs function as “large area heaters” capable of rapid heating. The $14 \times 19 \text{ mm}$ design is selected as it fits the device geometry while maintaining the optimal resistance range (5–9 Ω).

The smaller Area Heaters (10×11 mm to 14×15 mm, Figures 3.12(C)–(F)) exhibited high initial resistances ($> 15 \Omega$). Due to the limited power generation ($P = V^2/R$), these heaters failed to reach the required temperature range. For instance, the 10×15 mm and 10×11 mm designs achieved maximum temperatures below 50°C , which is insufficient for LAMP amplification. Conversely, larger Area Heaters (18×19 mm and 14×19 mm, Figure 3.12(A) and (B)) consistently demonstrated rapid heating and stable equilibrium profiles. Both designs effectively lowered the resistance to a level where the 3.7 V battery could drive the reaction temperature to the required range. All samples in these categories exhibited a rapid thermal response, reaching equilibrium within 2 minutes. This fast warm-up time is advantageous for point-of-care applications, minimising the total time-to-result.

Since both large configurations are capable of heating effectively, the final selection was based on the specific resistance value rather than area alone. The results indicate that heaters with an initial resistance between 5Ω and 9Ω reliably achieve the target LAMP temperature of 60 – 65°C . Heaters with resistance below this range tend to overheat (exceeding 65°C), while those above it fail to reach the threshold.

It is also important to address why larger heater dimensions (e.g., 30×50 mm) were not pursued. First, the standard width of our microfluidic device is around 15 mm. A heater significantly wider than the chip would result in inefficient heat dissipation to the surroundings rather than the sample. Second, increasing the area further would reduce resistance to a point where the equilibrium temperature would far exceed the LAMP requirements, resulting in amplification failure.

3.1.8 Thermal Uniformity and Surface-to-Liquid Temperature Correlation

To ensure successful nucleic acid amplification, the heating element must provide a spatially uniform thermal field, avoiding localised hot spots that could denature enzymes or cold spots that would inhibit the reaction. To evaluate this, the thermal spatial distribution of five randomly selected prototype heaters was characterised using infrared (IR) thermography.

Figure 3.13 presents the steady-state thermal images and corresponding dual-axis temperature profiles for the selected heaters. Despite minor variations in fabrication dimensions, all heaters exhibited a characteristic “flat-top” temperature profile. The horizontal (width) and vertical (length) profiles confirm that the central active region (specifically the 10×10 mm area corresponding to the reaction chamber location) maintains a high degree of uniformity. The thermal gradients are effectively confined to the outer edge of the printed electrodes, ensuring that the entire reaction volume is subjected to a consistent thermal environment.

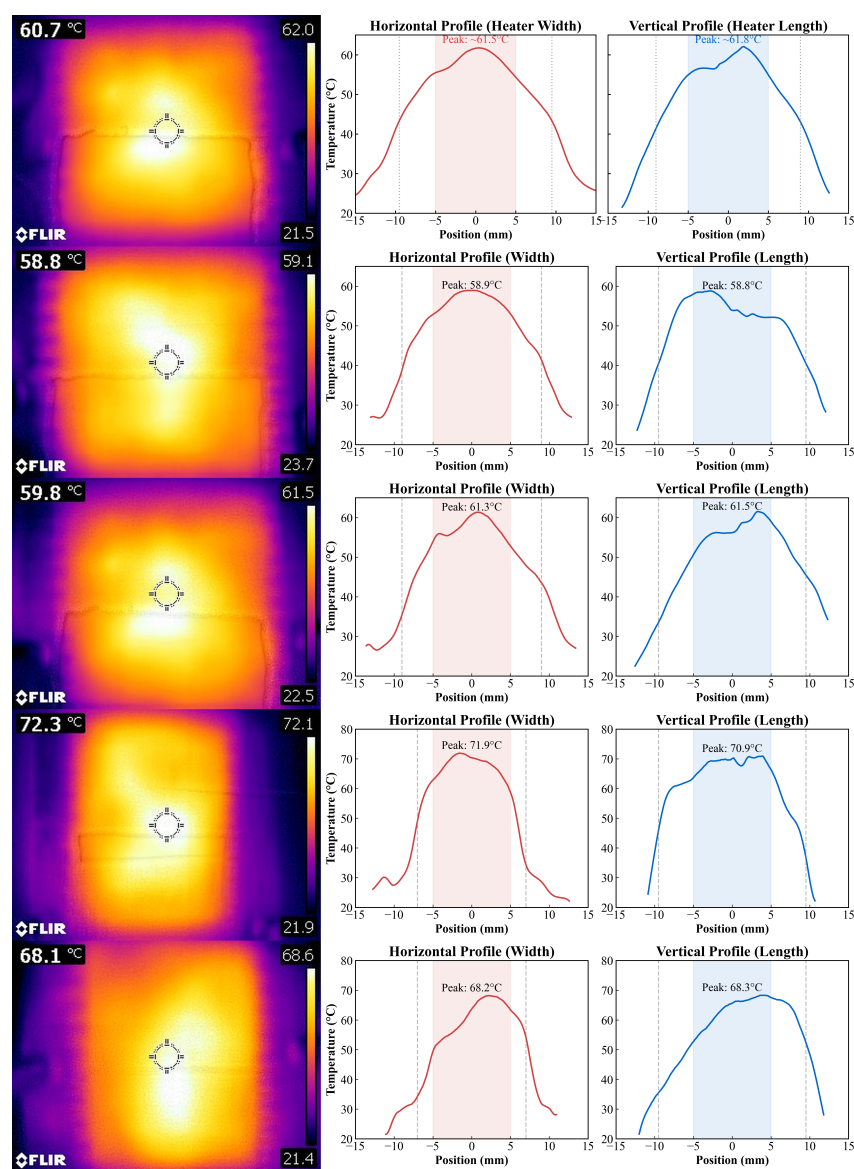


Figure 3.13: **Thermal uniformity analysis of five randomly selected PTC heaters.** The left panels show the steady-state IR thermal images, and the right panels display the corresponding horizontal (red) and vertical (blue) temperature profiles. All samples demonstrate a stable, uniform temperature plateau across the central reaction zone.

While IR thermography provides a first look for surface uniformity and peak intensity, it measures the temperature of the bare heater surface ($T_{surface}$). In the actual LAMP device, the microfluidic chamber is placed in direct contact with this surface. Consequently, the actual temperature of the liquid sample (T_{liquid}) differs from the surface reading due to two physical factors: Thermal Resistance: Heat loss occurs during transfer through the sealing film and into the liquid; Thermal Distribution: The thermal mass of the fluid and the device acts as a low-pass filter, further smoothing the temperature distribution via surface contact.

Experimental comparison revealed that the equilibrium liquid temperature is consistently 5–6 °C lower than the peak surface temperature measured by the IR camera. For instance, the fourth and fifth heaters in Figure 3.13 exhibited peak surface temperatures of approximately 72 °C and 68 °C, respectively. However, simultaneous thermocouple monitoring (corresponding to 5.6 Ω , 9.5 Ω in Figure 3.12) revealed that the water inside the chamber reached equilibrium temperatures of 66 °C and 63 °C.

This correlation confirms that heaters showing slightly elevated surface temperatures under IR inspection actually deliver the optimal physiological range (60–65 °C) to the biological sample. Therefore, while IR thermography validates the manufacturing quality and spatial uniformity of the printed traces, the in-chamber thermocouple measurements (as detailed in Section 2.5.6) remain the standard for verifying the biochemical operating conditions.

3.1.9 Operational Stability and Reusability

To validate the robustness of the printed IDE-PTC heaters for point-of-care diagnostics, we evaluated their performance under three critical conditions: long-term continuous operation, repeated thermal cycling, and extended shelf-life storage. These tests ensure that the printed conductive network remains stable without degradation due to electromigration, thermal expansion stress, or environmental oxidation over time.

The ability to maintain a constant temperature throughout the reaction duration (30–60 minutes) is essential for isothermal amplification. Figure 3.14(A) illustrates the temperature profile of the heater powered continuously for 60 minutes at 3.7 V. The device exhibited a rapid thermal response, reaching the target setpoint of 63.5 °C within 180 seconds. Once steady state was achieved, the temperature remained highly stable, with fluctuations confined to ± 0.25 °C for the majority of the operation. A minor increase in fluctuation amplitude (± 0.8 °C) was observed

in the final 10 minutes. It is possibly due to the slight variations in ambient convection, while the temperature remains well within the tolerance for LAMP reactions. Crucially, no significant drift was observed, confirming that the heating element does not suffer from obvious resistance change under 1 hour load.

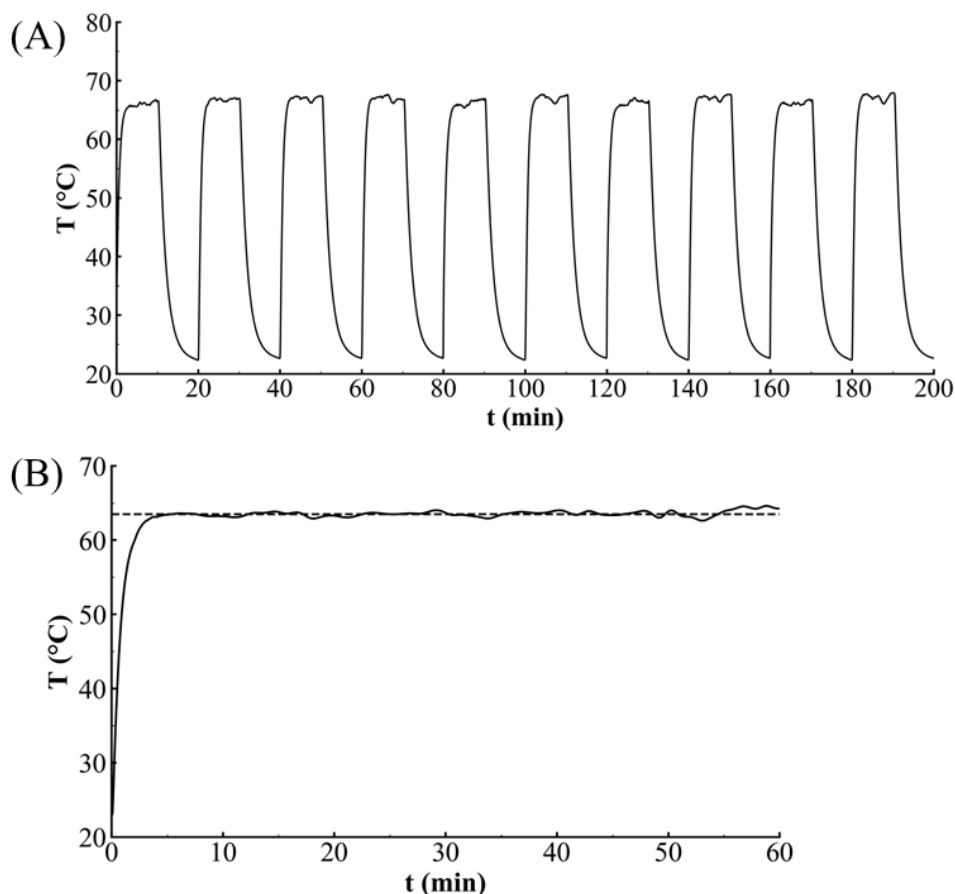


Figure 3.14: **Operational stability and reusability of the printed heater.** (A) Continuous heating profile over 60 minutes, showing a stable plateau at 63.5 °C with minimal fluctuation. (B) Cyclic heating test consisting of 10 consecutive ON/OFF cycles, demonstrating consistent thermal performance and mechanical durability.

To assess mechanical and electrical durability, the heater was subjected to 10 consecutive heating-cooling cycles (ON for 10 min, OFF for 10 min). As shown in Figure 3.14(B), the heating profiles were consistent across all 10 loops. The heating rate and peak plateau temperature remained reproducible, with no significant hysteresis or performance decay observed between the first and tenth cycles. This indicates that the repeated thermal expansion and contraction did not undermine the performance of the PTC heater, verifying the device's potential for reusability.

Finally, the long-term chemical stability of the printed traces was evaluated by comparing the performance of two randomly selected devices immediately after fabrication (“Fresh”) and after 9 months of storage under ambient conditions. Figure 3.15 presents the in-chamber liquid temperature profiles for both devices. The aged heaters exhibited heating curves nearly identical to their fresh state, achieving similar equilibrium temperatures ($\sim 63\text{--}66\text{ }^{\circ}\text{C}$). This result confirms that the IDE-PTC heaters are chemically stable and resistant to oxidation, ensuring a shelf life of at least 9 months without the need for special packaging.

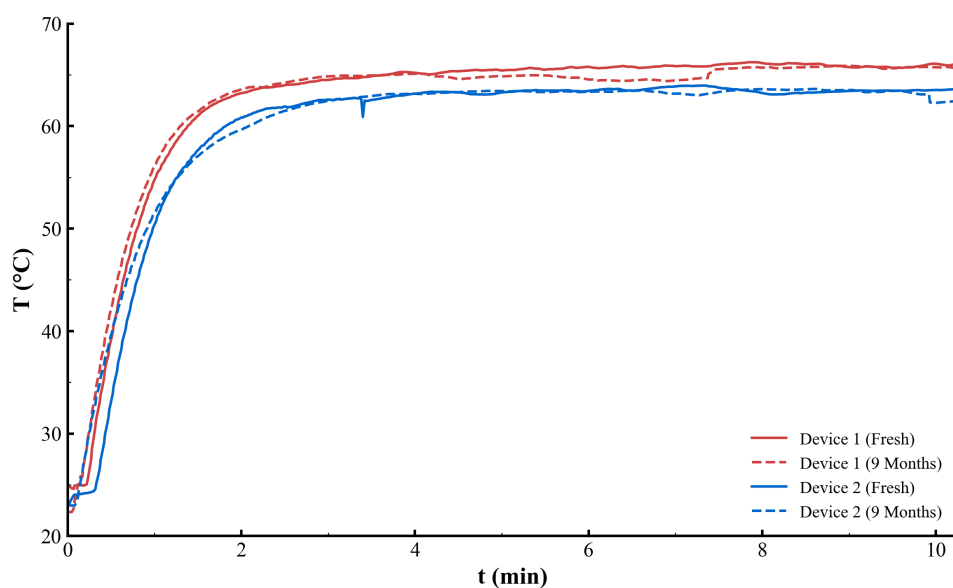


Figure 3.15: **Shelf-life stability analysis comparing the heating performance of two devices immediately after fabrication and after 9 months of storage.** The overlapping profiles indicate negligible degradation in electrothermal efficiency.

3.1.10 Power Consumption and Energy Efficiency

For field-deployable nucleic acid testing, energy efficiency is a critical parameter as it dictates the device’s operating time on portable battery power. We evaluated the power consumption of the optimal heater ($14 \times 19\text{ mm}$, in-device equilibrium temperature around $66\text{ }^{\circ}\text{C}$) powered by a single 3.7 V lithium-ion battery (2600 mAh). The current profile, $I(t)$, was monitored during the standard 40-minute LAMP reaction window, encompassing both the initial heating and temperature stabilisation phases.

The total energy consumption per reaction, $E_{reaction}$, was calculated by integrating the instantaneous power over the reaction duration ($t_{reaction} = 40$ min):

$$E_{reaction} = \int_0^{t_{reaction}} P(t) dt = V_{supply} \int_0^{t_{reaction}} I(t) dt \quad (3.6)$$

where V_{supply} is the constant battery voltage (3.7 V).

As shown in Figure 3.16, the Positive Temperature Coefficient (PTC) property of the heater results in a self-regulating current profile. At startup ($t = 0$), the low room-temperature resistance induces a peak current of ~ 0.47 A, facilitating a rapid thermal rise. As the heater reaches its operating temperature, the resistance increases, causing the current to decay and stabilize at a steady-state value of ~ 0.15 A within 10 minutes.

Substituting the measured current profile into Equation 3.6, the total energy consumption for a single reaction is calculated to be approximately 381 mWh, corresponding to an average power draw of 0.57 W. Given the total energy capacity of a standard 2600 mAh lithium-ion battery ($E_{batt} \approx 9620$ mWh), the theoretical number of reactions per charge is:

$$N_{runs} = \frac{E_{batt}}{E_{reaction}} \approx \frac{9620 \text{ mWh}}{381 \text{ mWh}} \approx 25.2 \quad (3.7)$$

Thus, a single charge can support over 25 complete amplification cycles, confirming that the device is highly energy-efficient and suitable for handheld, battery-powered operation in resource-limited settings.

3.1.11 Micro-scale IDE Fabrication and Characterisation

While the manual stencil printing method successfully produced functional heaters, the resolution of the finger gap was limited to approximately 0.5 mm. To explore the theoretical benefits of micro-scale gaps (specifically the potential for reduced resistance and higher power density), a collaboration was established with Jeff Kettle's group, which is specialising in high-resolution metal micro-patterning.

The micro-scale interdigitated electrodes (IDEs) were fabricated using a "Growth-and-Transfer" additive manufacturing process. Unlike traditional subtractive wet etching, this method utilises a sacrificial aluminium carrier substrate coated with a photolithographically patterned photoresist. Zinc is selectively electroplated into the developed patterns to form high-resolution

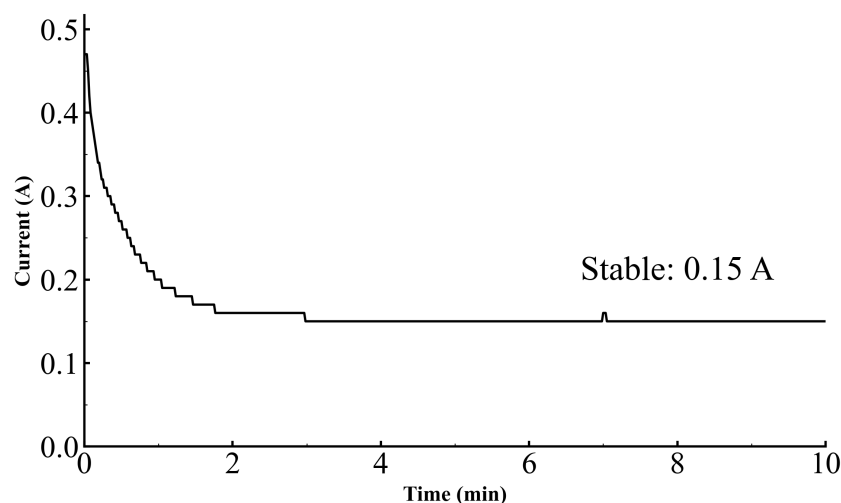


Figure 3.16: **Current consumption profile of the printed heater powered by a 3.7 V source.** The PTC effect generates an initial high-current surge for rapid heating, followed by a self-regulated drop to a stable baseline (~ 0.15 A), minimising energy waste during the plateau phase.

conductive tracks. These zinc tracks are subsequently transferred onto the polylactic acid (PLA) substrate, embedding them flush with the surface to ensure mechanical robustness. This additive technique allows for the creation of precise electrode fingers with gaps as small as $50\ \mu\text{m}$, significantly finer than the limits of mechanical stencils (Harwell *et al.* 2025).

To evaluate the viability of this technology, a set of sample IDEs was obtained with varying active areas and gap sizes: 10×10 mm heating area with $100\ \mu\text{m}$ gap, 4×4 mm heating area with $100\ \mu\text{m}$ and $50\ \mu\text{m}$ gaps. The PTC ink was applied over these zinc IDEs, and the devices were cured according to the standard protocol. Figure 3.17 illustrates the dimensions of the fabricated zinc IDEs.

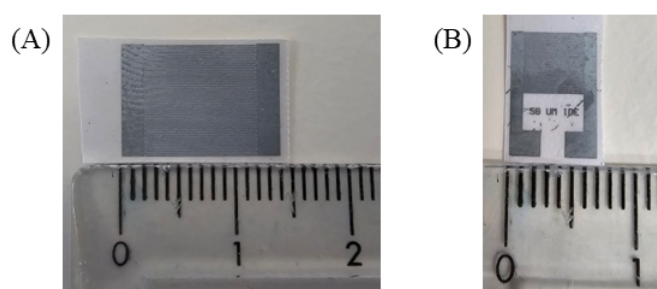


Figure 3.17: **Dimensions of the micro-scale zinc interdigitated electrodes.** (A) 10×10 mm IDEs. (B) 4×4 mm IDEs. The zinc electrodes were patterned via wet etching on a paper substrate, achieving gap sizes as small as $50\ \mu\text{m}$.

The electrical and thermal performance of these prototypes was initially characterised under a 5 V DC supply. The results are summarised in Table 3.6. A significant variance in performance was observed. The larger 10×10 mm heaters exhibited extremely high resistances (ranging from 360Ω to nearly $3 \text{ k}\Omega$), likely due to the insufficient PTC ink coating. In contrast, the smaller 4×4 mm heaters demonstrated more promising characteristics, with the $50 \mu\text{m}$ gap variants achieving resistances as low as 100Ω .

Table 3.6: **Electrical and thermal characteristics of micro-scale zinc PTC heaters under a 5 V supply.** The 4×4 mm design with a $50 \mu\text{m}$ gap (Sample 4#) exhibited the lowest resistance and highest temperature, while the larger 10×10 mm design showed unexpectedly high resistance.

Group	Sample	Gap (μm)	Initial R (Ω)	Current (A)	T_{eq} ($^{\circ}\text{C}$)
10×10 mm	1#	100	360	0.04	65.3
	2#	100	1450	0.03	64.9
	3#	100	1650	0.02	56.5
	4#	100	2830	0.02	44.2
4×4 mm	1#	100	250	0.02	71.5
	2#	50	174	0.01	63.2
	3#	50	128	0.01	69.0
	4#	50	100	0.02	76.6

Sample 4# from the 4×4 mm group ($50 \mu\text{m}$ gap, 100Ω) was selected for further testing against the three power sources identified in Section 3.1.5. The heater reached equilibrium temperatures of 70.6°C under the 3 V DC supply and 75.5°C with the 3.7 V Lithium-ion battery.

Notably, the heater also achieved 62.9°C when powered by the 3 V button battery. This result is particularly interesting given the failure of the button battery in previous macro-scale tests (Section 3.1.5). In this specific case, the high resistance of the micro-heater (100Ω) matched better with the internal resistance of the button battery, minimising the internal voltage drop and allowing a larger proportion of the power to be delivered to the heater. While this suggests that button batteries could be viable for high-resistance micro-heaters, the overall heating area of 4×4 mm remains too small for the proposed heater design.

Although the 4×4 mm micro-heaters demonstrated high temperatures, their active area was insufficient to cover the reaction chamber of the proposed LAMP device, which requires a length of at least 10 mm. Consequently, the investigation focused on optimising the 10×10 mm configuration. A new batch of 10×10 mm zinc IDEs with $50 \mu\text{m}$ and $100 \mu\text{m}$ gaps was fabricated and carefully coated with the PTC ink.

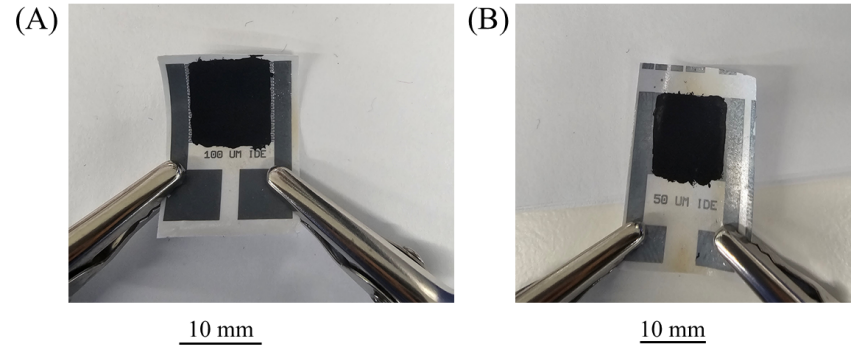


Figure 3.18: **Fabricated 10×10 mm micro-scale PTC heaters.** (A) Heater with $100 \mu\text{m}$ gap zinc electrodes. (B) Heater with $50 \mu\text{m}$ gap zinc electrodes.

Figure 3.18(A) and (B) show the fabricated devices. The electrical resistance and thermal performance were evaluated under a 3 V DC supply (consistent with the button battery voltage). The results are listed in Table 3.7.

Table 3.7: **Electrical and thermal performance of 10×10 mm micro-scale heaters under a 3 V DC supply.** Despite the reduced gap sizes, the resistance remained high ($> 98 \Omega$), preventing the heaters from reaching the target temperature of 60°C .

Gap Size	Sample	Resistance (Ω)	T_{eq} ($^\circ\text{C}$)
$50 \mu\text{m}$	1#	106	52.9
	2#	98	54.8
	3#	115	51.6
$100 \mu\text{m}$	1#	125	50.6
	2#	144	50.3
	3#	129	50.6

Contrary to the theoretical expectation that reducing the gap size reduces resistance, the 10×10 mm micro-heaters exhibited consistently high resistance values, ranging from 98Ω to 144Ω . The heating power generated at 3 V ($P \approx 0.09 \text{ W}$) was insufficient, resulting in equilibrium temperatures between 50°C and 55°C , which falls below the threshold required for LAMP amplification.

In conclusion, while micro-scale IDEs offer theoretical advantages, the electrical properties of the zinc substrate impose a limit on the maximum effective heating area. To achieve the required power for the 10×10 mm size, the macro-scale silver printing method remains the superior approach due to the higher conductivity of the silver traces.

3.2 Discussion

3.2.1 Deviations from Idealised Geometric Scaling

A critical evaluation of the experimental data reveals a systematic divergence from the idealised 2D thin-film resistance model typically used to design interdigitated electrodes ($R = R_s \cdot L/W_{eff}$). While this theoretical framework correctly predicts the general inverse relationship between heater footprint and bulk impedance, the absolute measured values and scaling behaviour deviate significantly due to the physical realities of the stencil printing process and the composite nature of the PTC ink.

Theoretically, based on the manufacturer's nominal sheet resistance of $20 \text{ k}\Omega/\text{sq}$ and an electrode gap of 0.5 mm, the calculated resistance for the largest configuration ($18 \times 19 \text{ mm}$) was projected to be approximately 30.9Ω . However, the measured average value was consistently lower (7.7Ω). This significant reduction occurs because the stencil-printing process deposits a substantially thicker active layer than the ultra-thin films assumed in standard sheet resistance characterisation. Because sheet resistance is inversely proportional to thickness ($R_s = \rho/t$), the increased ink volume effectively creates a 3D bulk resistor, advantageously lowering the total device impedance to a level compatible with 3.7 V battery operation (Perelaer *et al.* 2010).

Furthermore, the relationship between the electrode gap and the resulting resistance was found to be strictly non-linear. Assuming ideal geometry, doubling the electrode gap (L) from 0.5 mm to 1.0 mm should theoretically double the resistance (from 9.8Ω to 19.6Ω). However, the measured resistance for the 1.0 mm gap was 23Ω , representing an increase of over 130%. This discrepancy is attributed to the slump and spreading effects inherent to the deposition of wet conductive pastes. After the stencil is removed, the silver ink spreads slightly before thermal curing (Campos-Arias *et al.* 2024). A constant lateral spread of even 0.1 mm per electrode edge drastically alters the proportional ratio of the actual physical gaps. A nominal 0.5 mm gap is physically reduced to a much smaller effective channel than a nominal 1.0 mm gap subjected to the same absolute spread. Consequently, resistance drops exponentially faster as nominal gaps are reduced, confirming 0.5 mm as the optimal design choice.

In addition to gap resolution, the manual application of the squeegee introduced variations in the effective thickness and packing density of the PTC layer. Although the PTC layer thickness was not precisely controlled during fabrication, approximate caliper measurements showed that the total stack thickness increased from approximately 0.09 mm for the uncoated paper substrate to approximately 0.11 mm after silver deposition, and to approximately 0.20–0.30 mm after PTC deposition and curing. This indicates that the dried PTC coating contributed an approximate additional thickness of 0.09–0.19 mm above the silver-coated paper. These process-dependent variations help explain observed anomalies such as certain 14×19 mm heaters exhibiting lower resistance than some larger 18×19 mm counterparts. In manual stencil printing, it is challenging to maintain perfectly uniform pressure across differing spatial footprints. A slightly thicker deposition or firmer packing of the PTC ink over a 14×19 mm area can readily produce a lower effective resistance than a thinner or less uniform coating spread across a larger 18×19 mm area.

This geometric penalty is further corroborated by the area-scaling analysis shown in Figure 3.11(B). For an ideal two-dimensional film with fixed sheet resistance and $L = 0.5$ mm, the resistance can be approximated as

$$R = \frac{0.5R_s}{(W_a - 1)L_o} \quad (3.8)$$

which can be further simplified to $R \propto A^{-1}$ when W_a is sufficiently large. However, power-law regression of the experimental data yielded a steeper exponent of -1.63 . Furthermore, as the heater dimensions decreased, the standard deviation between replicates increased substantially, for example $\pm 21.5 \Omega$ for the 10×11 mm group.

These phenomena are intrinsically governed by the microstructural evolution of the conductive percolation network within the PTC composite. The PTC composite conducts through a three-dimensional continuous network formed by carbon particles dispersed within a polymer matrix. As the physical dimensions of the resistor are miniaturised, the structural stability of this percolation network becomes increasingly vulnerable to microscopic variations. Specifically, non-uniform interparticle spacing, localised void formation, and variation in effective coating thickness can create bottlenecks in the conductive pathways. In small areas, these microscopic defects lead to disproportionately high resistance and low reproducibility. Conversely,

larger geometries, such as 18×19 mm and 14×19 mm, facilitate the establishment of a more robust co-continuous structure. This macroscopic scaling mitigates the impact of localised voids and filler agglomeration, ensuring more abundant three-dimensional conductive pathways and a more stable conductive network (Dong *et al.* 2025).

Ultimately, while the IDE geometry provides the necessary theoretical framework for low-voltage operation, the absolute thermal performance is governed by these physicochemical interactions and deposition dynamics. Recognising these 3D physical constraints justifies the final design selection: the minimum feasible gap (0.5 mm) leverages the spreading effect to maximise conductivity, while the larger footprints mitigate percolation defects and manual printing variations, guaranteeing a reproducible thermal output for point-of-care amplification.

3.2.2 Electrothermal Mechanism and Performance

Precise thermal control is the prerequisite for successful Loop-Mediated Isothermal Amplification (LAMP). To ensure optimal Bst polymerase activity without enzyme denaturation, the reaction temperature must be strictly maintained within the 60–65 °C window. In this work, we employed a manual stencil printing technique to fabricate disposable IDE-PTC heaters. By systematically optimising the trace geometry (specifically the length and width ratios defined in the mask design), we demonstrated the ability to precisely tune the initial resistance (R_0), thereby achieving in-device equilibrium temperatures ranging from 35 °C to 70 °C to suit various diagnostic requirements.

The thermal stability of these stencil-printed devices is driven by the positive temperature coefficient effect of the composite ink. Mechanistically, this phenomenon arises from the thermal expansion of the polymer binder matrix, which increases the tunnelling distance between conductive fillers and disrupts the percolation network upon heating. (K. Liu *et al.* 2023, Choi *et al.* 2021)

According to the manufacturer's specifications (Celanese Corporation 2025), this specific PTC formulation can achieve an absolute maximum switching temperature of approximately 85 °C when driven by higher voltages (e.g., 10 V) at a 0.5 mm gap. In our optimised system, the surface temperature successfully plateaus at approximately 70 °C, which safely delivers the

required 60 – 65 °C to the liquid sample. It must be acknowledged that at the 3.7 V maximum supplied by the portable lithium-ion battery, the heater does not reach the absolute physical saturation point of the PTC material. However, rather than being a limitation, this operating state represents a highly advantageous dynamic equilibrium.

Because heating power is dictated by the relationship $P = V^2/R$, operating at a lower voltage (3.7 V) means that as the device heats up and the resistance begins to climb the steep slope of the R-T curve, the power generation drops rapidly. The system quickly reaches a point where the heat generation perfectly matches the heat dissipation to the surrounding environment at 70 °C.

We experimentally verified this mechanism in Section 3.1.4, where the electrical characterisation of heaters with varying geometries revealed a non-linear Resistance-Temperature (R-T) relationship. As the temperature approached the target amplification range, the measured resistance exhibited a sharp increase. This dynamic resistance shift creates an intrinsic negative feedback loop: as the heater warms, the increasing resistance naturally throttles the current. This was clearly observed in the power consumption profile (Section 3.1.10), where the initial surge current (~ 0.47 A) rapidly decayed to a stable steady-state (~ 0.15 A). This self-regulation effectively “locks” the temperature at the setpoint of 63.5 °C without the need for complex external controllers.

To contextualise the performance of our device, it is necessary to benchmark it against current mainstream and emerging heating strategies.

1. **Printed Circuit Board (PCB) Heaters** (H. P. Kim *et al.* 2025, K. P. Jiang *et al.* 2024, Papadakis *et al.* 2022): Currently widely chosen for portable devices, etched copper PCBs offer robustness but suffer from high thermal mass and zero self-regulation. They required bulky feedback circuitry and temperature sensors to prevent thermal runaway.
2. **Chemical Heating** (Liao *et al.* 2026, Mauk *et al.* 2024): Some instrument-free platforms have utilized exothermic chemical reactions. While electricity-free, these methods lack fine thermal control and often require phase-change materials to buffer temperature spikes, complicating the device architecture.

3. **Printed Microheaters (Z. Jeroish *et al.* 2023, Byers *et al.* 2020):** Thin metal traces (Ag, Au, Cu) printed on a solid or film substrate. Some microheaters were reported to achieve high temperatures at sub-volt driving conditions. However, such designs typically rely on low-resistance metallic traces and require precise external control to prevent thermal overshoot. For isothermal amplification, particularly LAMP, stable temperature maintenance within a narrow range (63–68 °C) is more critical than rapid or high-temperature heating.
4. **High-Voltage Integrated Carbon/Metal Heaters:** Other composite heaters, such as the carbon-paste integrated chips (Nguyen *et al.* 2023), successfully achieve amplification temperatures but often operate at higher voltages (9 V), necessitating multiple batteries or step-up converters, which increase the complexity.
5. **Field-Induced Heating (Xie, Q. Jiang, *et al.* 2024, Ahn *et al.* 2024:** Non-contact strategies utilise alternating magnetic fields to induce eddy currents or employ plasmonic nanomaterials to convert light into heat. While they achieve extremely rapid heating rates, these methods require bulky high-frequency power supplies or complex optical arrangements that severely hinder portability. Furthermore, dispersing photothermal nanoparticles directly into the reaction mixture can quench fluorescence signals (Phuong *et al.* 2026), complicating optical detection.

In contrast to these approaches, the heater developed in this study combines the advantages of low-power operation and simplified control. By optimising the formulation and stencil pattern, we achieved a device that operates on a single 3.7 V lithium-ion battery with a power consumption of only 0.57 W. This offers a significant advantage over PCB or Peltier-based systems in terms of energy efficiency, while the inherent PTC safety mechanism eliminates the need for additional control electronics required by traditional resistive heaters.

3.2.3 Operational Robustness and Device Sustainability

A critical requirement for the field deployment of any point-of-care diagnostic device is its operational robustness against ambient temperature fluctuations. It is essential to ensure that a device calibrated in a controlled 25°C laboratory environment can still function reliably across diverse global climates, ranging from cooler environments (e.g., 18°C) to extreme tropical con-

ditions (e.g., 35°C). Traditional constant-voltage resistive heaters are highly vulnerable to such ambient shifts, as their heat generation is fixed; a drop in ambient temperature inevitably leads to a proportional drop in reaction temperature, requiring complex feedback controllers to adjust the power.

Our printed PTC heaters overcome this limitation through intrinsic self-regulation. To demonstrate this environmental tolerance, the device's thermal profile was evaluated across a severe ambient temperature gradient (the whole device was set in the corresponding environment for 10 mins before the test): a 4°C cold room, a 25°C standard room, and a 37°C incubator (Figure 3.19). Remarkably, a 33°C shift in ambient conditions only resulted in a 6°C shift in the steady-state plateau (from 61°C to 67°C). This occurs because the PTC material actively compensates for the environment: at 4°C, greater convective heat loss prevents the heater from immediately reaching its switching threshold, forcing it to remain in a lower-resistance, higher-power state to automatically generate more heat. Conversely, at 37°C, the heater rapidly reaches its resistance spike, which limits current and automatically reduces the power output to prevent thermal runaway.

Consequently, for standard moderate fluctuations (e.g., transitioning between an 18°C indoor environment and 28°C outdoor conditions), the resulting equilibrium temperature will remain safely locked within the 60–65°C tolerance window of the Bst polymerase. No hardware, power, or design changes are required. For extreme deployments, such as equatorial climates consistently exceeding 35°C, the modularity of the design provides a simple solution. Rather than redesigning the entire microfluidic cassette, operators can simply substitute a "tropical" heater variant (a heater with a higher initial resistance that plateaus at a slightly lower temperature) to ensure universal applicability.

Beyond environmental tolerance, a commercially viable device must endure prolonged storage and rigorous operation without performance degradation. A common failure in printed electronics, particularly those utilising silver nanoparticles, is the gradual oxidation or sulphurisation of the conductive tracks upon exposure to ambient air, which leads to unpredictable resistance drift over time (D. U. Kim *et al.* 2016). In this study, we demonstrated the exceptional chemical stability of the stencil-printed graphene-silver heaters.

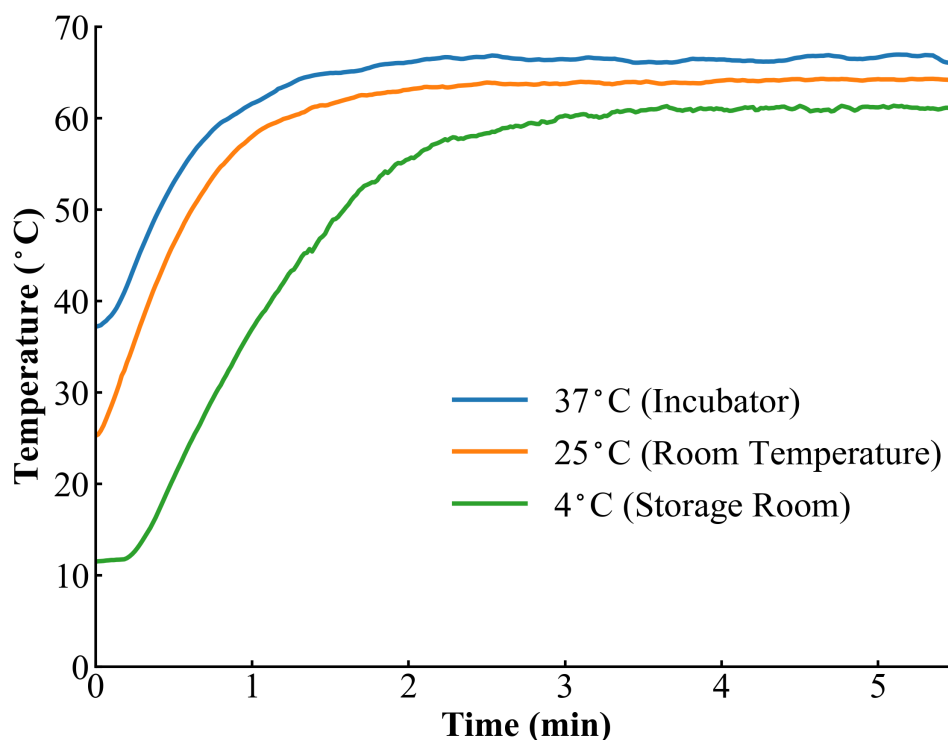


Figure 3.19: **Thermal profile of the PTC heater operating under varying ambient conditions.** The device was tested in a 4°C cold room, a 25°C standard room, and a 37°C incubator. Despite a 33°C fluctuation in the surrounding environment, the steady-state equilibrium temperature shifts by only ~6°C (plateauing between 61°C and 67°C). This demonstrates the intrinsic self-regulating capability of the PTC material, which actively compensates for environmental heat loss to maintain the operational temperature window for LAMP amplification without external feedback controllers.

By comparing the heating performance of devices immediately after fabrication and after 9 months of ambient storage (Section 3.1.9), we found no noticeable difference in their thermal profiles. Both “fresh” and “aged” heaters achieved similar equilibrium temperature and heating rate. This long-term stability suggests that the PTC coating layer effectively encapsulates the silver trace, creating a barrier against environmental moisture and sulfur, and thus preventing the formation of non-conductive silver sulfide (Ag_2S) layers that typically undermine the performance of silver circuits (Lv *et al.* 2023). Therefore, our devices can be stored for extended periods without the need for vacuum packaging or desiccant pouches, significantly simplifying logistics for low-resource settings.

Furthermore, the device must withstand the mechanical stresses induced by repeated thermal expansion and contraction. In our cyclic heating experiments, the heater maintained consistent performance across 10 consecutive heating-cooling loops. The absence of delay or resistance drift indicates strong interfacial adhesion between the composite ink and the paper substrate. Thus, the mechanical integrity of our heating devices was proved, demonstrating their ability to withstand continuous heating cycles.

Finally, true sustainability in field diagnostics is defined by both energy independence and carbon footprint reduction. Standard benchtop thermal cyclers consume 100–500 W and rely on stable grid power or heavy, emission-producing generators. In contrast, our electrical characterisation confirmed that the PTC device consumes only 0.57 W per run. This ultra-low power requirement means a single standard 2600 mAh lithium-ion battery can support over 25 complete LAMP reactions. From a life-cycle perspective, this architecture significantly reduces the carbon emissions associated with diagnostic testing. Traditional PCB-based heaters rely on FR4 fibre-glass substrates and subtractive copper etching—processes associated with notably high global warming potentials, the use of toxic brominated flame retardants (BFRs), and the generation of hazardous electronic waste (Harwell *et al.* 2025). By transitioning to an additive printing process on a biodegradable cellulose matrix and operating at sub-watt power levels, this platform minimises both the manufacturing carbon footprint and the generation of end-of-life electronic waste, establishing a highly sustainable choice for global point-of-care diagnostics.

3.2.4 Fabrication Challenges and Future Outlook

The optimised stencil-printed heater presented in this work was the result of an iterative design process that highlighted critical trade-offs between material properties, geometric resolution, and fabrication accessibility. Retrospectively, our initial attempts (Section 3.1.2) utilising pure PTC ink suffered from high resistance. Conversely, IDT-PTC heaters offered excellent resistance and heating performance but failed in cost control. While the hybrid silver IDE and PTC composite successfully balanced these properties (Section 3.1.3), the fabrication process revealed some challenges, particularly the electrode geometry and ink rheology.

Due to its high viscosity, the PTC ink proved unsuitable for standard mesh-based screen printing, necessitating a manual stencil printing approach. The difficulty in controlling the squeegee pressure and speed manually inevitably leads to slight variations in the effective thickness and packing density of the deposited PTC layer between batches. Since the PTC thickness was not independently controlled in this work, it should be treated as a process-dependent source of variation rather than a deliberately tuned design parameter. These coating variations are likely to be a major contributor to the resistance distribution observed in our results, where equilibrium temperatures varied between 50 and 70 °C across different fabrication batches for the 18 × 19 mm and 14 × 19 mm heater dimensions.

However, these fabrication limitations essentially constitute the device's greatest strength: accessibility. The fabrication workflow developed here avoids the need for sophisticated clean-room facilities, photolithography, or high-precision screen printers. The shadow masks were produced using a simple commercial vinyl cutter (Silhouette Portrait® 2) with a resolution of 0.5 mm, and the conductive silver traces were deposited manually. This indicates that functional, self-regulating molecular diagnostic heaters can be manufactured in low-resource settings, or even “at home”, using minimal infrastructure. This ease of fabrication is a critical step toward enabling local production of diagnostics in developing regions, bypassing the supply chain logistics often required for mass-produced PCB heaters.

To strengthen the consistency of heaters, we explored micro-scale Interdigitated Electrodes (IDEs) fabricated via chemical etching of zinc-coated PET. While 4×4 mm designs functioned correctly, scaling up to 10×10 mm resulted in heating failure (3.1.11). This size-dependent failure can be attributed to the limited conductivity of the thin etched zinc traces compared to the bulk silver paste used in macro-scale designs.

Measurements indicated a trace resistance of approximately 6Ω along the length of the busbars. In the larger 10×10 mm design, the current is forced to travel long, narrow zinc fingers to reach the active heating gap. Consequently, the metal fingers effectively act as resistors connected in series with the PTC ink. This creates a significant internal voltage drop, reducing the effective potential applied across the heating material towards the centre of the heater. In addition, at the micro-scale, the likelihood of etching defects (e.g., disconnected fingers) increases with the total area. A 10×10 mm area contains significantly more potential failure points than a 4×4 mm area, contributing to the variability and higher average resistance.

Looking forward, two distinct pathways exist for the evolution of this technology.

1. **Industrial Scale-Up:** To reduce batch-to-batch variation, the transition to semi-automatic screen printing or Roll-to-Roll (R2R) manufacturing is necessary. This would require specialised printing equipment capable of handling high-viscosity PTC inks and improving the reproducibility of coating deposition. Rather than using thickness as a deliberately tuned parameter, future manufacturing should minimise uncontrolled variation in effective dry-film thickness, packing density, and ink distribution. In future studies, coating thickness should be quantified using profilometry, cross-sectional microscopy, or mass-per-area measurements so that its contribution to resistance variation can be separated from the effects of IDE gap and active area.

2. **Advanced IDE Arrays:** Despite the initial failure of the zinc-based micro-IDEs with the dimension of 10×10 mm, the architecture remains a promising direction if implemented with highly conductive printed silver traces or with smaller dimensions. A future goal is to fabricate arrays of IDE-PTC heaters. Such a system could enable simultaneous multiplexed amplification of different targets or facilitate complex fluidic operations, such as the sequential melting of wax valves, by activating specific heating zones on demand.

3.3 Contribute to knowledge

This chapter addresses the critical challenge of thermal management in point-of-care molecular diagnostics. By moving away from traditional, power-consuming benchtop equipment, we have established a robust framework for fabricating low-cost, energy-efficient, and self-regulating heaters. The specific contributions to the field are summarised as follows:

We identified and quantified the limitations of applying standard 2D thin-film resistance equations to manually printed 3D composite heaters. By analysing the effects of wet-ink spreading on micro-scale gaps and the finite-size scaling of percolation networks in small footprints, we established a non-linear power-law scaling relationship ($R \propto A^{-1.63}$). This analytical framework serves as a practical design guide for future researchers, enabling IDE heater geometries to be approximately scaled for low-voltage operation and target temperature windows without relying exclusively on exhaustive empirical trial-and-error.

We successfully formulated and optimised a stencil-printed composite heater that overcomes the limitations of single-component materials. By combining silver IDEs with PTC ink, we balanced the high electrical conductivity of silver with the mechanical flexibility and chemical inertness of PTC ink. This approach resolved the problems observed in pure silver paste heaters and the excessive resistance found in pure PTC ink paste, yielding a robust device capable of sustaining the 60–65 °C equilibrium temperature required for LAMP.

We demonstrated that the PTC effect intrinsic to the composite ink can be exploited as a passive control mechanism. Unlike standard PCB or polyimide heaters that require active PID control and temperature control components, our device self-regulates its temperature. Furthermore, the stability tests indicated that the heater retained its thermal performance under the

tested ambient-temperature conditions; the material naturally compensates for external heat loss, maintaining the target amplification window even when subjected to severe ambient temperature fluctuations (from 4 °C to 37 °C). This reduced the required circuit complexity and supported operation across the tested ambient-temperature range.

We established a geometric optimisation to tune the heater resistance for low-voltage operation. With a total power consumption of approximately 0.57 W and an energy efficiency allowing for over 25 reaction cycles per charge of a 3.7V lithium-ion battery, this work provides a potential route for handheld diagnostics in resource-limited settings where grid power is unavailable. We also proved that our heaters can resist environmental oxidation for at least 9 months under ambient conditions and withstand the mechanical stress of 10 thermal cycles without performance degradation. These findings confirm that manually stencil-printed devices can meet the stability standards required for commercial shelf-life and field deployment.

Development of Multiplexed Lateral flow Strips and Optimisation of On-Paper Isothermal Amplification

This chapter details the design, fabrication, and optimisation of the multiplexed lateral flow strips. The experimental workflow begins with the evolution of the strip architecture from line format to dot format (Sections 4.1.1–4.1.3), followed by the systematic optimisation of critical parameters, including membrane selection, buffer composition, and surface blocking in Section 4.1.4. A significant portion of this section is dedicated to resolving the conjugate depletion phenomenon that the high yield of isothermal amplification suppresses the control line by establishing a balanced labelled primer ratio.

Subsequently, the feasibility of integrating the amplification reaction directly onto a paper substrate is evaluated (Sections 4.1.7–4.1.8). This investigation characterises the interactions between DNA amplicons and porous matrices, explicitly comparing the trapping and releasing properties of glass fibre and cellulose filter paper. Finally, the optimised on-paper amplification is validated within a prototype PMMA microfluidic chamber, demonstrating the successful thermal and fluidic integration required for the final device described in Chapter 5.

4.1 Results

4.1.1 Validation of LAMP Assays

This project aims to detect and differentiate five major respiratory viruses: Influenza A (Inf A), Influenza B (Inf B), Respiratory Syncytial Virus A (RSV A), Respiratory Syncytial Virus B (RSV B), and SARS-CoV-2 (COVID). Since these pathogens cause similar clinical symptoms but require different medical interventions, the specificity of the amplification assay is critical. Before integrating the reaction into the paper-based device, the performance of each LAMP assay was verified using a standard real-time qPCR machine.

The validation experiments followed the protocols described in Section 2.1 and 2.2. Each 25 μL reaction contained 15 μL of master mix, 5 μL of primer mix, and 5 μL of DNA template (10^5 copies/reaction). Figure 4.1 presents the amplification results for all five targets.

Unlike PCR, which relies on discrete thermal cycling, LAMP is an isothermal process where amplification occurs continuously. While the real-time thermocycler software records fluorescence data in 'cycles' (with one reading taken every 60 seconds), this represents the reaction duration. Therefore, throughout this study, the Time to Threshold (T_t), which is defined as the time in minutes required for the fluorescence signal to exceed the background baseline, is used as the quantitative metric for amplification speed, replacing the traditional Cycle Threshold (C_t).

The real-time amplification plots (Figure 4.1, left column) demonstrate that all five assays successfully generated robust fluorescence signals. RSV A, RSV B, and COVID assays exhibited rapid exponential growth with early T_t values (indicating fast reaction kinetics). The Inf A and B assays also showed clear amplification, reaching saturation within the 30-minute reaction window, although minor variations in T_t were observed between these two subtypes and their replicates.

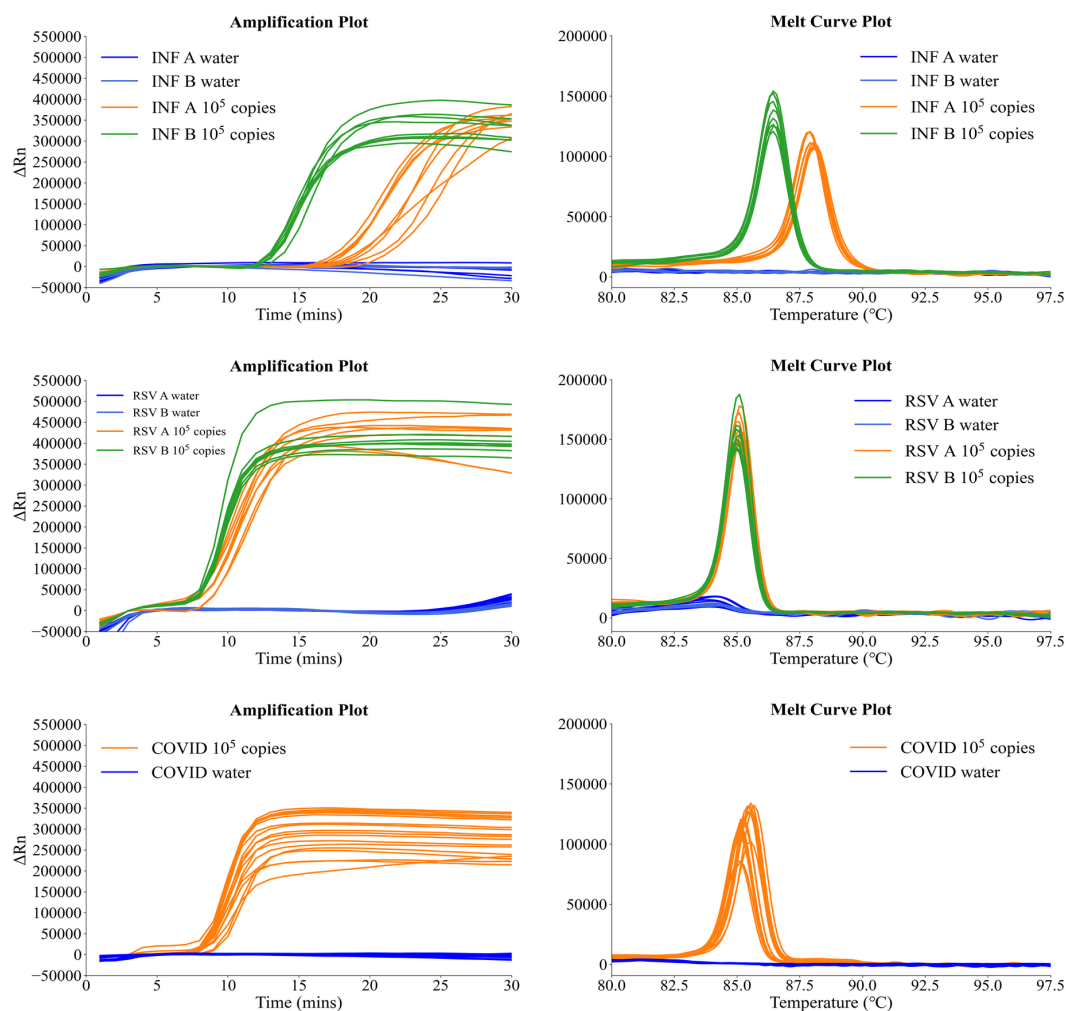


Figure 4.1: **Validation of LAMP assays for respiratory virus targets using real-time fluorescence monitoring and post-amplification melt curve analysis.** The left column displays real-time amplification plots (fluorescence intensity vs. cycle number), showing signal accumulation over 30 cycles (30 min). The right column displays the corresponding melt curve plots (derivative of fluorescence $-\Delta Rn/\Delta T$ vs. temperature), indicating the dissociation temperature (T_m) of the amplicons. The panels represent: (Top row) Influenza A (purple) and Influenza B (red); (Middle row) RSV A (purple) and RSV B (red); (Bottom row) SARS-CoV-2 (COVID). All reactions used 10^5 copies/reaction of the synthetic template. The successful amplification curves and the presence of single, distinct melting peaks for each reaction confirm the sensitivity and specificity of the primer sets.

To verify reaction specificity and ensure the absence of non-specific products such as primer-dimers, a post-amplification melt curve analysis was performed (Figure 4.1, right column). The derivative plots show the melting temperature (T_m) at which the double-stranded amplicons dissociate. The presence of single, sharp peaks confirms specific amplification, while the distinct T_m values indicate potential for discriminating these subtypes based on thermal profiles. Both the amplification and the melt curves show that the selected LAMP assays are functional, efficient, and highly specific for their intended targets.

4.1.2 Analytical Limit of Detection of LAMP Assay

To determine the analytical LoD of the LAMP assay, a ten-fold serial dilution was performed using the Influenza B synthetic DNA template. While the ultimate goal is a pentaplex system, Inf B was selected as the representative model for detailed Limit of Detection (LoD) characterisation. Because the biochemical mechanism across all five targets is identical, each assay utilises the same core reagents (LAMP master mix) optimised under a unified thermal protocol (65°C). The real-time validation (Figure 4.1) also indicated that the Inf B primer set exhibits intermediate amplification kinetics, showing the average performance.

As detailed in Table 4.1, the template solution was diluted in nuclease-free water, starting from a stock concentration of 2.5 ng/ μ L down to a theoretical 1 copy/reaction. The calculation was done in Section 2.1.2. Amplification was assessed using two methods: Real-time LAMP, which offers quantitative insights, and In-tube LAMP, which serves as a convenient method for generating amplicons for subsequent lateral flow strip (LFS) detection.

Table 4.1: **Protocol for the preparation of Influenza B DNA template serial dilutions.** For clarity and direct comparison with sensitivity data, the mass concentration (0.02 ng/ μ L, equivalent to 0.1 ng per reaction) is also expressed as copies per reaction (copies/reaction), calculated based on the specific molecular weight of each synthetic template (see Table 2.1).

Solution	Concentration	Preparation
A	2.5 ng/ μ L	Original template (300 ng) + 120 μ L water
B	0.1 ng/ μ L	8 μ L A + 192 μ L water
C	0.02 ng/ μ L (equals to 4.12×10^8 copies/reaction)	80 μ L B + 320 μ L water
D	10^7 copies/reaction	24.3 μ L C + 975.7 μ L water
E	10^5 copies/reaction	10 μ L D + 990 μ L water
F	10^4 copies/reaction	100 μ L E + 900 μ L water
G	10^3 copies/reaction	10 μ L E + 990 μ L water
H	10^2 copies/reaction	10 μ L F + 990 μ L water
I	10 copies/reaction	10 μ L G + 990 μ L water
J	1 copy/reaction	10 μ L H + 990 μ L water

The real-time amplification curves for varying template concentrations are presented in Figure 4.2(A). A clear correlation was observed between the template concentration and the Time to Threshold (T_t), with the T_t value increasing as the copy number decreased. The assay demonstrated consistent positive amplification from 10^5 down to 10^2 copies/reaction. At the lower limit of 10 copies/reaction, only two out of four replicates showing amplification, while the 1 copy/reaction samples and the No Template Controls (NTC) yielded no significant fluorescence signal.

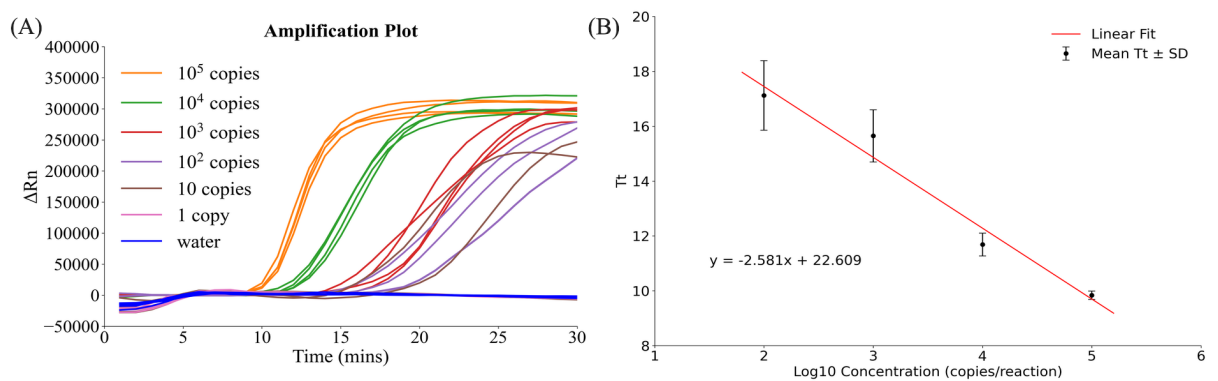


Figure 4.2: **LoD analysis of the LAMP assay using Influenza B template dilutions.** (A) Real-time amplification plots showing fluorescence intensity (ΔRn) over time. Robust amplification curves are observed for template concentrations ranging from 10^5 to 10^2 copies/reaction. At the limiting dilution of 10 copies/reaction, part of the amplifications were valid (2/4 replicates positive), while 1 copy/reaction and the No Template Control (water) remained below the threshold. (B) Standard curve derived from the mean T_t values of reliable replicates (10^5 to 10^2 copies), showing a decreasing trend of T_t when the concentration increases. Error bars represent standard deviation ($n = 4$).

To quantify the Limit of Detection (LoD), a standard curve was constructed by plotting the mean T_t values against the logarithm of the template concentration for the consistently positive replicates (10^5 to 10^2 copies), as shown in Figure 4.2(B). The linear regression analysis is performed to observe the trend. The following equation is calculated:

$$T_t = -2.346 \times \log_{10}(C) + 21.588 \quad (4.1)$$

where C represents the template concentration (copies/reaction). Based on the standard curve and the standard deviation of the lowest reliable concentration, the theoretical LoD was calculated to be approximately 18 copies/reaction.

Following quantitative analysis, the compatibility of the LAMP assay with commercial lateral flow strips (detecting FITC-labelled DNA) was evaluated. Figure 4.3 compares the LFS results of real-time LAMP products and in-tube LAMP products obtained. The real-time LAMP was conducted in a qPCR machine, and in-tube LAMP was conducted in an Eppendorf tube in a heat block at 65°C. Consistent with the real-time fluorescence data, the LFS results for the Real-time LAMP products showed clear test lines from 10^5 down to 10^2 copies, with partial detection at 10 copies and no signal at 1 copy. Similarly, the In-tube LAMP reaction conducted in a heat block without real-time monitoring demonstrated reliable detection down to 10^2 copies/reaction, with negative results observed at 10 and 1 copies.

The comparison indicates that there is no order-of-magnitude difference in LoD between the highly controlled Real-time LAMP and the simplified In-tube LAMP. Consequently, the In-tube method, with a practical LoD of 100 copies/reaction, was selected for the subsequent examination of lateral flow strips due to its simplicity and ability to generate sufficient amplicon quantities. It is worth noting that while this LoD is sufficient for proof-of-concept, the primary objective of this project is not to optimise the LAMP assay for ultra-sensitive detection, but rather to advance the integration of nucleic acid amplification with paper-based detection platforms.

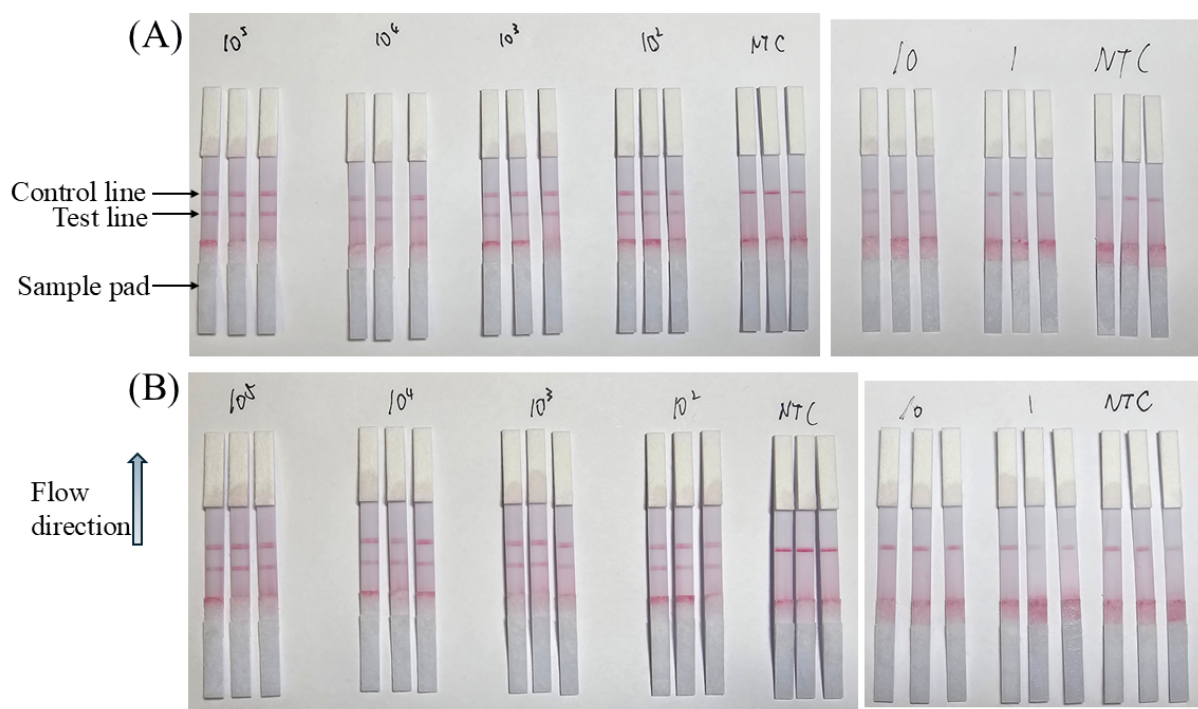


Figure 4.3: **Comparison of Lateral Flow Strip (LFS) detection limits for Influenza B amplicons.** (A) LFS results using products from the Real-time LAMP reaction, showing consistent positive test lines down to 10^2 copies. (B) LFS results using products from the In-tube LAMP reaction, showing a similar detection limit of 10^2 copies/reaction. The presence of the control line validates the proper flow of the strip in all tests, while the test line (T) indicates positive amplification. NTC: No Template Control.

4.1.3 Development of Multiplexed Lateral Flow Strips

Respiratory tract infections caused by different viral pathogens often require distinct clinical management despite causing similar symptoms (Dabisch-Ruthe *et al.* 2012). Thus, the ability to simultaneously detect multiple targets within a single reaction (multiplexing) is highly desirable to reduce assay time, sample consumption, and overall cost compared to running parallel single-plex tests (Y. Jiang *et al.* 2025). The objective of this phase was to develop a pentaplex lateral flow strip capable of distinctively identifying Influenza A, Influenza B, RSV A, RSV B, and SARS-CoV-2 amplicons.

Traditional lateral flow strips typically employ a line format for test and control zones. While robust for single or dual-target applications, scaling this format to accommodate five distinct test lines within the standard dimensions of a lateral flow membrane presents significant challenges. Closely spaced lines can lead to issues with signal resolution and potential cross-reactivity due to the diffusion of reagents between adjacent capture zones (Mohd Hanafiah *et al.* 2017). To overcome these spatial constraints and minimise interference, a micro-dot array strategy was adopted for this project. The dot format allows for higher-density patterning, ensuring sufficient physical separation between capture antibodies specific to different targets while maintaining a compact strip size suitable for integration into a portable device (Hofmann *et al.* 2021, Anfossi *et al.* 2018).

The general materials and buffer compositions utilised for strip fabrication were established in Section 2.4.3. However, fabricating multiplexed strips with precise dot arrays required a modification of the standard workflow to ensure accurate antibody distribution. In traditional lateral flow manufacturing (which uses continuous line formats), reagents are striped across a wide membrane card. The card is then fed into a strip cutter and sliced into individual 4 mm-wide strips. Because the reagent lines are continuous and perpendicular to the cut, slight lateral shifting or manual positioning variations during the cutting process do not affect the functionality of the final test lines.

Applying this standard method to a discrete dot array, however, is highly problematic for laboratory-scale prototyping. As illustrated in Figure 4.4, the dashed line indicates the intended starting path for the cutting blades across a large membrane. Because the starting point of a semi-automated strip cutter is rarely perfectly flush with the membrane edge, it varies slightly

depending on manual loading. If the pre-printed grid of micro-dots is laterally misaligned by even a fraction of a millimetre from this intended cutting line, the blades will shear directly through the capture zones, or the dots will end up severely off-centre. This manual variation can easily fail an entire manufacturing batch.

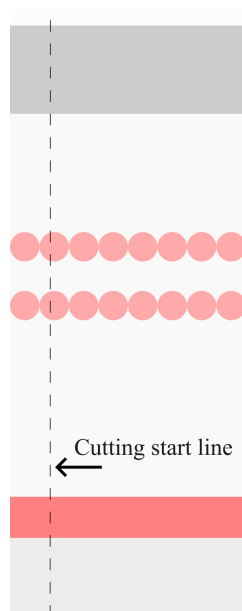


Figure 4.4: **Alignment challenges in dot-array lateral flow strip fabrication.** The schematic illustrates a large membrane card pre-printed with a dot array, where the dashed line represents the intended cutting path. Due to the high precision required, minor lateral misalignments during the manual loading of the cutter can cause the blades to shear directly through the capture zones, ruining the batch. To prevent this, a “cut-then-print” method was adopted for laboratory prototyping.

To prevent this misalignment and ensure reliable prototyping, the workflow was inverted to a “cut-then-print” approach. The blank nitrocellulose membranes were first cut into individual 4 mm-wide strips. These pre-cut strips were then secured in a dedicated strip holder on the BioSpot workstation, allowing the automated dispenser to accurately target the central axis of each narrow strip for reagent deposition. This pre-cutting step eliminates the risk of blade-shearing and guarantees perfectly centred capture zones.

The specialised multistep fabrication process adopted for these multiplex strips is illustrated in Figure 4.5. Initially, the capture antibodies corresponding to the five viral targets, alongside control antibodies, were dispensed in a predefined dot array onto the pre-cut nitrocellulose strips using the BioSpot workstation. To minimize non-specific binding and ensure uniform flow, the strips underwent a blocking step immediately after dispensing. They were immersed in a 1% BSA blocking solution and incubated on an orbital shaker for 1 minute, followed by a 5-minute

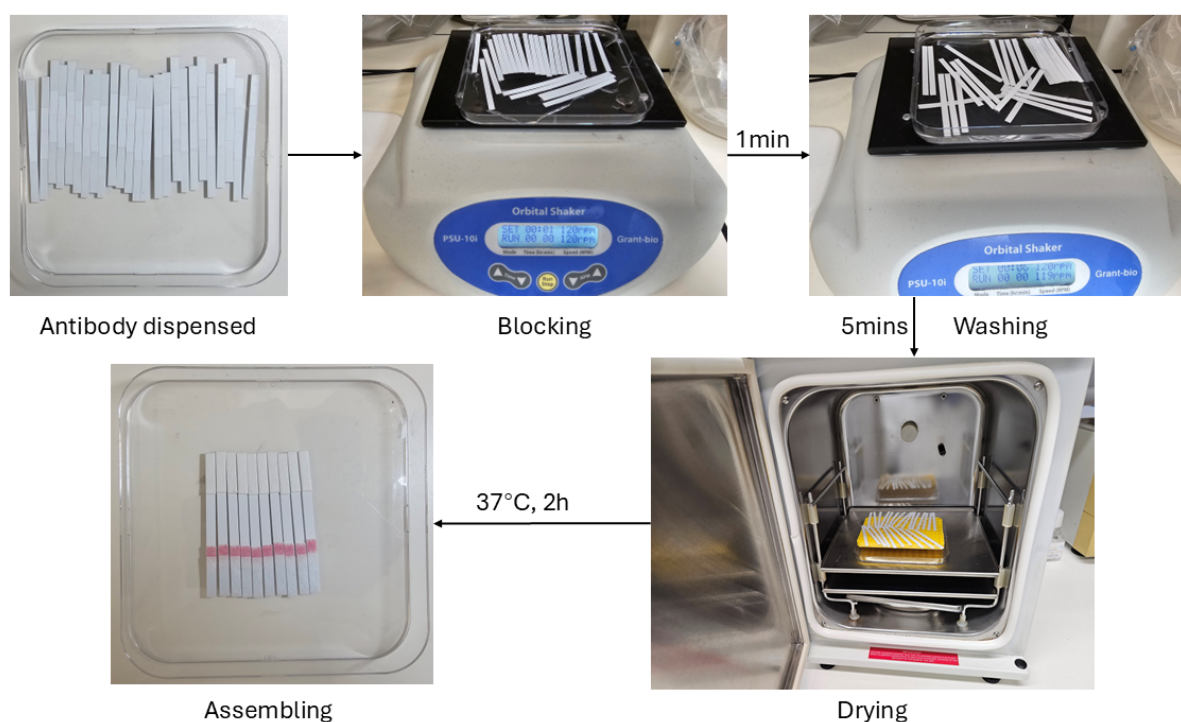


Figure 4.5: **The modified fabrication workflow adopted for producing multiplex dot-array lateral flow strips.** The process begins with the precise dispensing of capture antibodies onto pre-cut nitrocellulose strips. This is followed by a blocking step using 1% BSA on an orbital shaker (1 min), a washing step (5 min), and drying in an incubator at 37 °C. Finally, the treated membranes are assembled with the necessary pads to complete the multiplex strips.

wash with running buffer to remove excess blocking agent that could otherwise clog membrane pores. Subsequently, the strips were thoroughly dried in an incubator at 37 °C. The process concluded with the manual assembly of the treated membranes with the conjugate pad, sample pad, and absorbent pad to form complete lateral flow devices, ready for subsequent testing with multiplex LAMP products.

To assess the feasibility of the traditional layout, initial tests were conducted using a multi-line format where capture antibodies were dispensed as sequential bands along the nitrocellulose membrane. As illustrated in Figure 4.6, the performance of this configuration was compromised when multiple targets were present. Robust simultaneous detection is important, as respiratory viral co-infections (such as Influenza co-circulating with RSV or SARS-CoV-2) are frequently observed in clinical populations and can increase disease severity (Krumbein *et al.* 2022, Cong *et al.* 2022). A consistent signal depletion was observed, in which the binding of amplicons to the upstream test lines significantly reduced the availability of reagents for downstream capture

zones. This interference resulted in weaker signals for subsequent targets, particularly when high concentrations of multiple amplicons were introduced simultaneously. Such mutual weakening confirmed the limitations of the line format for this specific pentaplex application, necessitating a design that mitigates spatial competition.

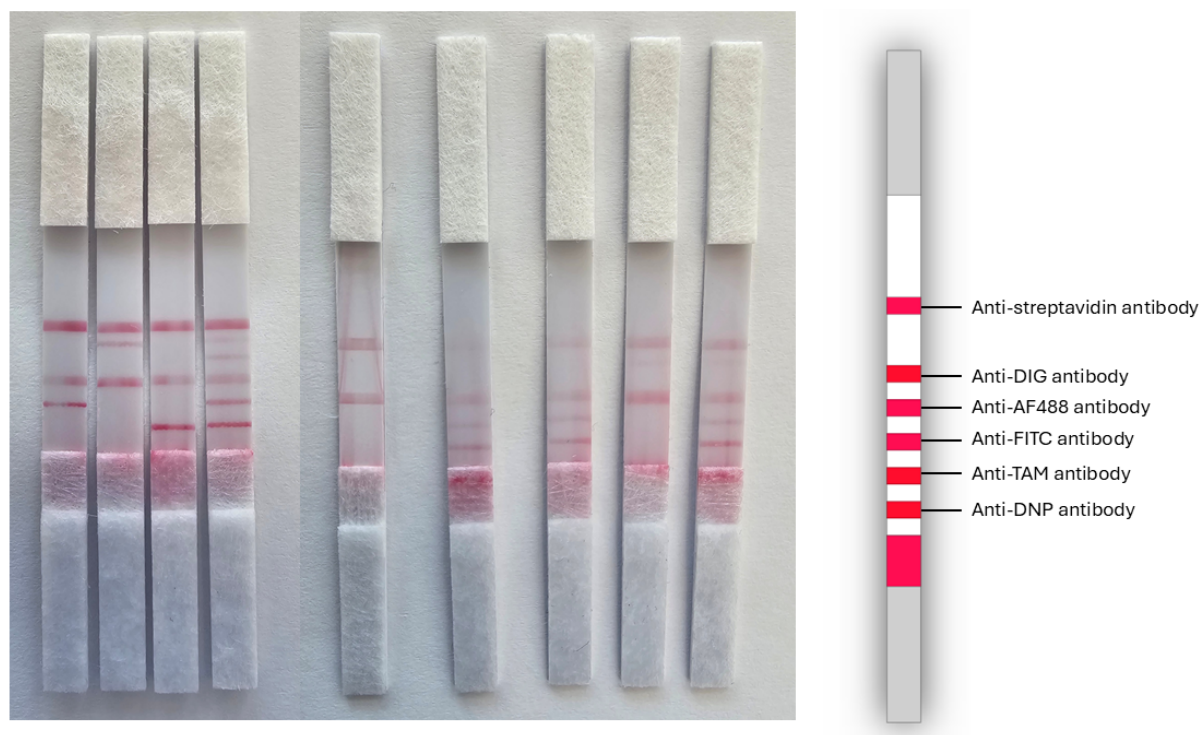


Figure 4.6: **Evaluation of the multi-line format for multiplex detection.** Random tests with various amplicon combinations reveal significant signal depletion in downstream test lines when upstream targets are present. This interference (mutual weakening) confirms the inadequacy of the sequential line configuration for simultaneous pentaplex detection.

In contrast, the micro-dot array configuration demonstrated superior performance by spatially segregating the capture zones, thereby reducing the shadowing effect seen in the line format. Figure 4.7 presents the validation results for the dot-array strips under various testing conditions, categorised by the complexity of the target mixture. The penta-plex scenario involved the simultaneous introduction of all five target amplicons (Inf A, Inf B, RSV A, RSV B, and COVID) into a single test, representing the most severe challenge for reagent competition and cross-reactivity. The triplex scenarios tested intermediate combinations (e.g., a mixture of COVID, Inf A, and RSV A) to simulate clinically relevant co-infection patterns. Finally, Single-plex sce-

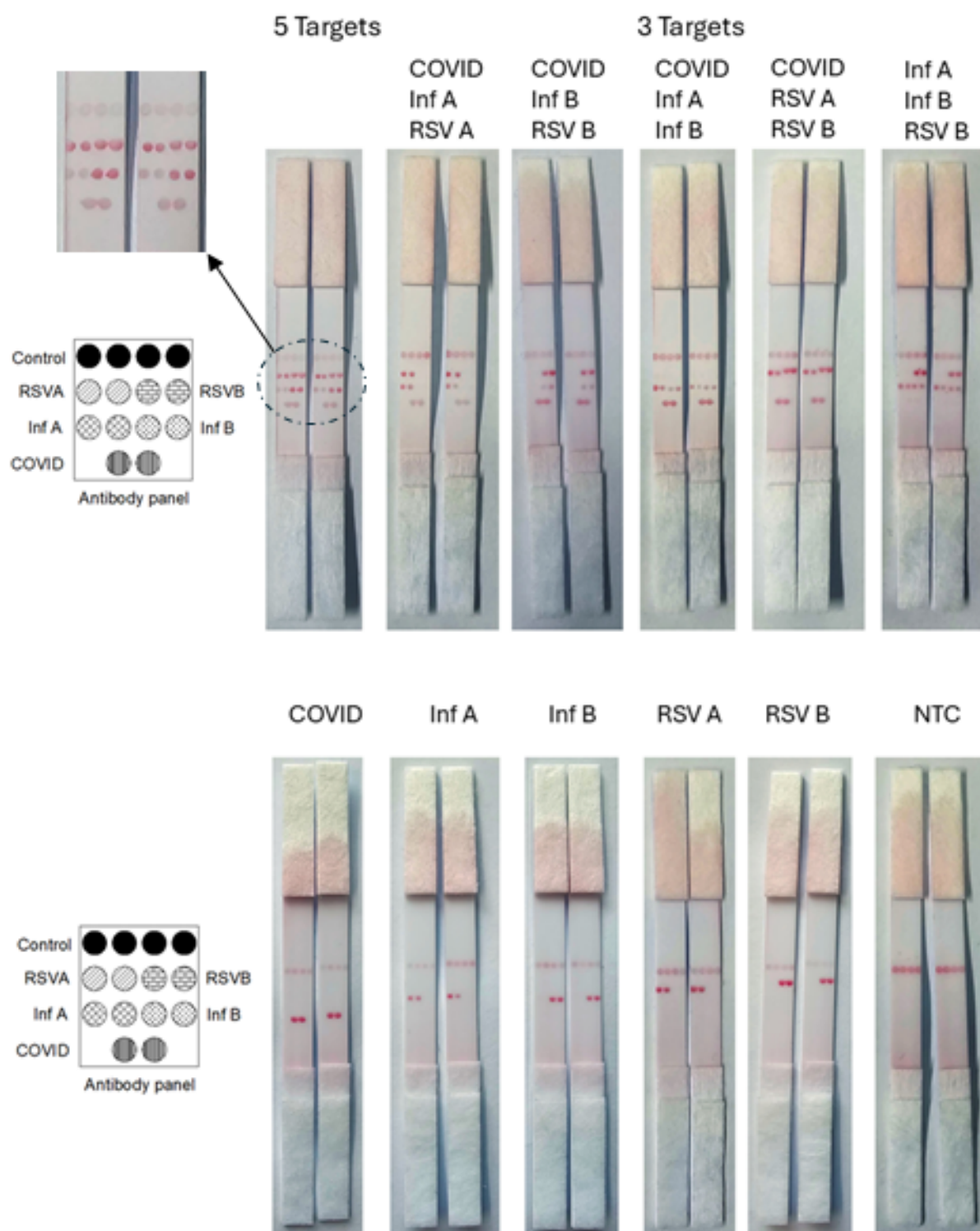


Figure 4.7: **Performance of the optimised micro-dot array lateral flow strips.** (Top) Detection of 5 targets (Pentaplex) and 3 targets (Triplex), showing clear and distinct signals for all present amplicons without downstream signal loss. (Bottom) Single-plex specificity tests for COVID, Inf A, Inf B, RSV A, and RSV B, along with a No Template Control (NTC). The specific alignment of positive dots corresponds to the antibody panel layout (right), confirming high specificity and the absence of cross-reactivity.

narios involved testing individual targets to verify the baseline specificity of each capture zone. In the penta-plex tests, distinct and robust signals were observed for all five targets. Notably, the detection of targets positioned further downstream, such as RSV A and RSV B, remained strong, indicating that the dot layout effectively preserves reagent availability across the strip.

Furthermore, the specificity of the assay was confirmed through selective testing. In triplex and single-plex experiments, only the relevant capture dots exhibited positive signals, with no visible cross-reactivity or false positives in the non-target zones. The No Template Control (NTC) strips showed only the control dots, validating the assay's background cleanliness. These results establish the dot-array format as the optimal configuration for the integrated device, offering both the LoD to detect multiple co-infecting pathogens and the specificity to distinguish between them without signal loss.

4.1.4 Optimisation of Multiplex Lateral Flow Strips

Developing the multiplexed lateral flow strip presented distinct challenges. Unlike the standard line format, the dot array introduced specific issues regarding flow uniformity and signal clarity. Weak signals and dirty backgrounds were frequent. Sometimes, the liquid front would simply stop, and the control lines would go missing. To resolve these issues, several critical components were optimised: the surface blocking treatment, the nitrocellulose membrane, the running buffer composition, and the LAMP assay conditions.

Surface Treatment and Membrane Selection

Background noise posed the most significant challenge. Without a blocking step, the gold nanoparticles adhered non-specifically to the empty nitrocellulose fibres, making the results unreliable (O'Farrell 2008). As shown in Figure 4.8, unblocked membranes exhibited high background signals, leading to false positives where negative controls appeared reactive. Furthermore, false negatives were occasionally observed, likely due to the instability of the unblocked capture dots. To address this, a blocking step using 1% BSA was introduced (Merck Millipore 2019). The improvement was important. The blocked membranes showed clean backgrounds with no false positives. The BSA effectively passivated the empty binding sites, preventing non-specific adsorption. However, this treatment introduced a trade-off. In lateral flow assays, surface treatments of nitrocellulose membranes can alter membrane surface chemistry and flow characteristics. Empirical data from LFA development (Valtion Teknillinen Tutkimuskeskus 2011)

shows that high concentrations of blocking agents (e.g., 3–5 % BSA with Tween-20) substantially increase wicking times compared with unblocked strips, indicating that blocking treatments can slow capillary transport while reducing non-specific binding. This increased flow resistance necessitated either larger sample volumes or longer reaction times, underscoring the importance of selecting a suitable membrane.

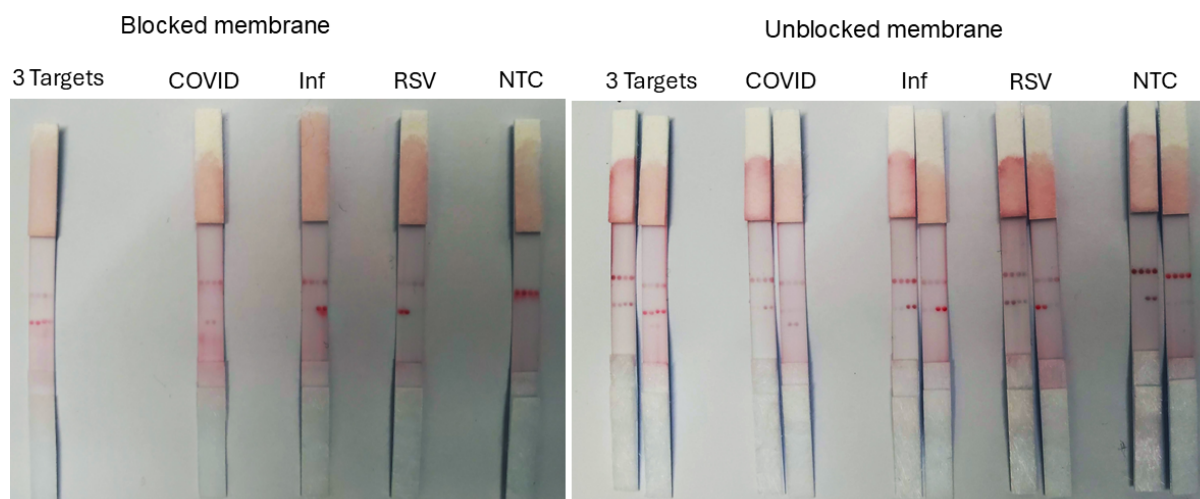


Figure 4.8: **Comparison of multiplex lateral flow strips with and without membrane blocking.** (Left) Blocked membranes show clean backgrounds and specific signal generation with no false positives. (Right) Unblocked membranes exhibit significant non-specific binding (red background haze), leading to false positives in the No Template Control (NTC) and potential false negatives due to signal loss.

Given the flow resistance caused by blocking, the intrinsic wicking rate of the membrane became a critical factor. Two commercial variants were evaluated after undergoing the same blocking protocol: FF120HP Plus and Hi-Flow Plus 90. Figure 4.9 compares their performance. The FF120HP Plus, known for its slower intrinsic flow rate, struggled significantly after blocking. Severe liquid stopping was frequently observed, where the sample front failed to reach the absorbent pad. This incomplete flow made the test invalid. The Hi-Flow Plus 90 membrane showed better wicking capability after BSA treatment. Its higher initial flow rate compensated for the resistance added by the blocking layer to some degree. Thus, the higher flow rate membrane was selected as a better choice for the blocked multiplex strips.

However, some of the high flow rate membranes still presented flow stopping, proposing the necessity of fine-tuning the blocking-washing procedure. It was found that the balance between blocking time and washing time directly influenced the final flow properties. Excessive blocking time or insufficient washing led to the accumulation of residual blocking agents. These

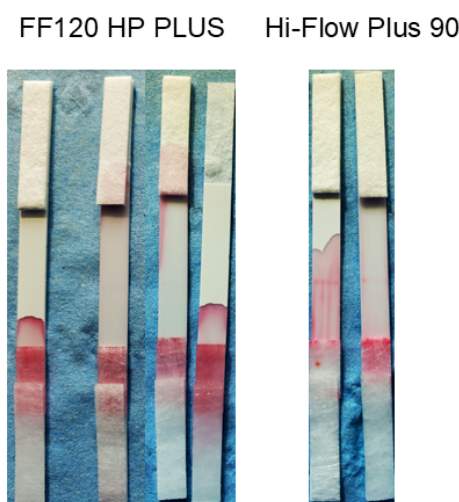


Figure 4.9: **Evaluation of flow performance for different nitrocellulose membranes following BSA blocking.** The slower-wicking FF120HP Plus membrane (left) exhibits severe flow stopping, with the liquid front failing to travel through the strip. The faster-wicking Hi-Flow Plus 90 membrane (right) shows better flow characteristics after blocking, facilitating sample migration.

residues could dry into crystals, physically clogging the membrane pores and further impeding flow. Relative to the brief time required for blocking, the washing step proved more demanding. The excess blocking agent relies on diffusion to exit the complex porous structure of the membrane and enter the wash buffer. Following established guidelines for lateral flow product development (Merck Millipore 2019), a protocol comprising 1 minute of blocking with 1% BSA followed by 5 minutes of washing was adopted. This duration was experimentally verified to be sufficient for effective surface passivation without compromising the membrane's capillary flow characteristics. Furthermore, the physical handling of the strips proved important. The immersion into the blocking solution had to be slow and deliberate. This allowed trapped air to be displaced from the pores, preventing dry spots. For the backed membranes used here, the solution should be wicked in from the edges before complete submersion. Ensuring a sufficient volume of blocking solution was also critical to completely cover the strips and provide enough mass of the blocking agent to coat the exposed polymer effectively (Sukumaran *et al.* 2020).

Running Buffer Composition

To ensure specific hybridisation and minimise background noise, the composition of the running buffer was then optimised. The running buffer serves two purposes: it must transport the reagents smoothly along the membrane and simultaneously facilitate the specific hybridisation between the target DNA and the capture probes. To achieve this, specific additives were incorporated into the buffer formulation. Tween 20, a non-ionic surfactant, was included to lower the

surface tension of the liquid. This surfactant promotes reagent dispersion and reduces the trailing of gold nanoparticles. Simultaneously, MgCl_2 was added to provide divalent cations (Mg^{2+}). These ions screen the negative charges on the DNA phosphate backbones, reducing electrostatic repulsion and thereby stabilising the hybridisation between the target amplicons and the capture probes (Jungbluth *et al.* 2025).

To identify the optimal base formulation, three different buffer systems were evaluated: deionised (DI) water, a TE-based buffer, and a PBS-based buffer (all containing the aforementioned additives). These specific bases were chosen to represent three different biochemical environments. DI water was included as a zero-salt baseline control to evaluate the intrinsic flow of the nanoparticles without any background ionic influence. TE buffer was selected because it is the universal standard for nucleic acid handling and storage, offering excellent pH stability for DNA amplicons. Finally, PBS was evaluated because it provides a physiological salt environment that is traditionally required to maintain the native conformation and maximum binding affinity of the antibodies and immobilised proteins used on the test strip.

As shown in Figure 4.10, the choice of buffer impacted assay performance. Initial tests using DI water demonstrated functional flow but suffered from a distinct background haze due to the lack of buffering capacity. The TE-based buffer proved unsuitable, inducing significant false positives likely caused by the destabilisation of the colloidal gold by EDTA. In contrast, the PBS-based buffer yielded the superior result, delivering strong, specific signals against a clean, white background. Thus, the PBS-based formulation was selected as the standard running buffer for all subsequent experiments.

Assay Conditions and Conjugate Balancing

A critical challenge encountered during the development of the multiplex LFS was the inconsistent appearance of the control line when running positive LAMP samples. In a valid lateral flow test, the control line must always appear to confirm proper liquid flow and reagent functionality, regardless of the presence of target DNA. Its absence renders the test invalid. We hypothesised that this phenomenon resulted from intense competition for the streptavidin-coated gold nanoparticles (SA-Au) used as the colourimetric reporter.

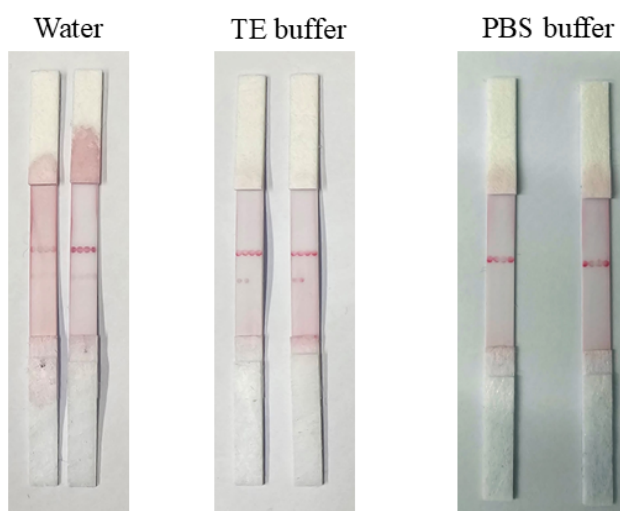


Figure 4.10: **Comparison of three running buffer formulations on multiplex strip performance.** (Left) Deionised water results in correct signal localisation but exhibits high background noise and trailing. (Center) TE-based buffer causes non-specific binding, leading to false-positive signals in the absence of target amplicons. (Right) PBS-based buffer (supplemented with 0.05% Tween 20 and 5 mM MgCl_2) delivers the optimal performance, showing clear specific signals with a clean background.

The visualisation of test lines relies on the high-affinity binding between biotinylated amplicons and the SA-Au conjugate as they migrate along the strip. LAMP amplification, however, is highly efficient, generating a large quantity of biotinylated amplicons. Furthermore, the reaction mixture inevitably contains residual biotinylated primers that did not participate in amplification. We hypothesised that this overwhelming excess of biotinylated molecules rapidly occupies all available binding sites on the SA-Au conjugate beads immediately upon release from the conjugate pad. Consequently, when the fluid front reaches the control line, which typically contains an anti-streptavidin antibody designed to capture SA-Au, insufficient free SA-Au remains to generate a visible signal.

To substantiate this hypothesis, an order-of-magnitude stoichiometric calculation was performed to compare the available streptavidin binding sites against the input biotin load. A standard LAMP reaction in this study utilises 0.25 μL of 20 μM biotinylated primers (with Inf B requiring 0.5 μL , 2.3). This equates to an initial input of 5.0 pmol to 10.0 pmol of biotin molecules per reaction.

To determine the binding capacity of the reporter, the quantity of functional binding sites on the gold conjugate was calculated. The lateral flow strips utilise 6 μL of 40 nm SA-Au conjugate at 10 OD. Based on the UV–vis quantification framework developed by Haiss (Haiss *et al.* 2007), a 1 OD suspension of 40 nm gold nanoparticles corresponds to a particle concentration of approximately 0.10 to 0.12 nM. Therefore, a 10 OD suspension provides a concentration of roughly 1.0 to 1.2 nM. Consequently, the 6 μL conjugate pad contains between 6.0 and 7.2 femtomoles (fmol) of gold nanoparticles.

The theoretical binding capacity of these nanoparticles can be estimated geometrically (D’Agata *et al.* 2017). A 40 nm spherical particle has a surface area defined by $S = \pi d^2 = \pi \times 40^2 \approx 5026 \text{ nm}^2$. Given that the streptavidin (SA) tetramer has a hydrodynamic diameter of roughly 5 nm, its projected footprint on the gold surface is $\approx 25 \text{ nm}^2$. While perfect tight-packing would theoretically allow ~ 200 molecules, realistic steric repulsion limits packing efficiency to 50–70%, resulting in a functional coating of 100 to 140 SA molecules per nanoparticle. Furthermore, due to the steric hindrance imposed by adsorption onto the spherical solid phase, a maximum of 2 out of the 4 binding pockets per SA tetramer remain sterically accessible to the target. This yields an estimated 200 to 280 biotin-binding sites per individual gold nanoparticle.

Multiplying the total nanoparticle yield (6.0–7.2 fmol) by the available sites per particle (200–280) gives a total theoretical binding capacity of approximately 1.2 to 2.0 pmol of biotin for the entire conjugate pad. When comparing this capacity (1.2–2.0 pmol) to the input biotin load (5.0–10.0 pmol), it is mathematically evident that the system is heavily oversaturated. Crucially, it must be noted that this 2.0 pmol capacity represents a highly optimistic upper bound. In a positive reaction, the binding entities are not small, free biotin molecules, but large, polymetric LAMP amplicons. When these bulky DNA concatemers bind to the nanoparticle, they induce severe steric shielding, physically blocking adjacent free streptavidin sites and substantially reducing the effective binding capacity well below the theoretical limit. Consequently, the high yield of biotinylated amplicons creates a severe stoichiometric excess that completely exhausts the gold conjugate at the test line, leaving no available SA-Au to migrate downstream to the control line.

To empirically confirm this calculated conjugate depletion, a series of investigative tests was conducted, as shown in Figure 4.11 ($n = 3$, the strips displayed serve as representative images of the consistent signal intensities and flow behaviours observed across all repeated trials). First, a baseline was established by running amplicons generated without any biotinylated primers. In

this scenario, the SA-Au conjugate had no targets to bind to in the test zone and migrated freely to the control line, resulting in a visible signal. Next, a blank LAMP reagent mix (containing biotinylated primers and master mix but no template) was run. The control line disappeared. This indicated that even without amplicons, the high concentration of unused biotinylated primers was sufficient to saturate the SA-Au conjugate or potentially inhibit the control line reaction. Another experiment was done by manipulating the gold nanoparticle concentration. When extra SA-Au conjugate was loaded onto the conjugate pad before running a standard positive sample, the control line reappeared, showing that increasing the supply of reporter particles could overcome the saturation. Furthermore, adding extra SA-Au after the initial sample had completely wicked through the strip resulted in a strong control signal. This demonstrated that the control line capture antibodies were not saturated or inhibited; the available SA-Au reporter complex had been depleted by upstream competition.

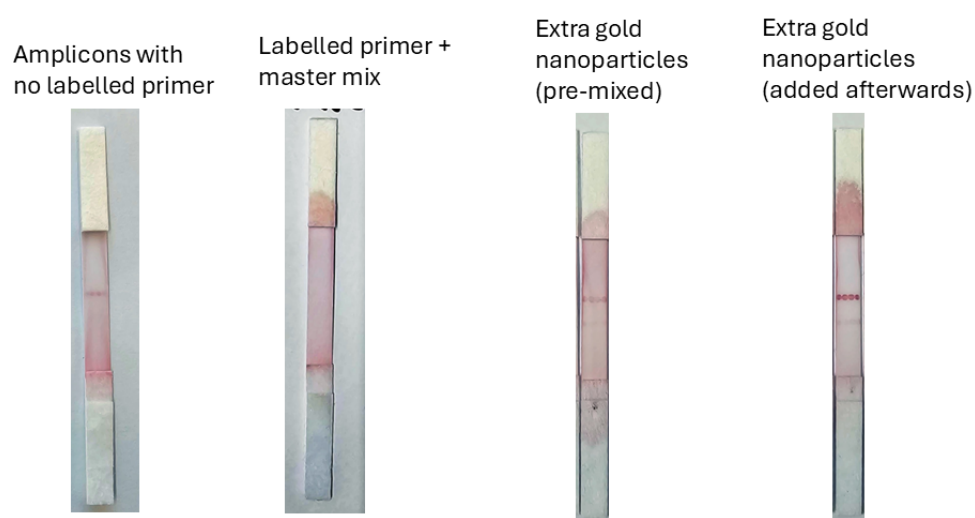


Figure 4.11: **Investigation into the mechanism of control line signal loss.** (From left to right) Running unlabelled amplicons results in a visible control line. Running blank LAMP reagents (containing biotinylated primers) causes the control line to disappear. Adding extra SA-Au conjugate before the run restores the control line. Adding extra SA-Au conjugate after the run generates a strong control line. These results confirm that the excess of biotinylated primers and amplicons saturates the SA-Au conjugate, depleting the supply available for the control line. The displayed strips are representative images selected from $n = 3$ independent experimental replicates, demonstrating highly consistent flow dynamics and signal capture across all trials.

Having established that an excess of biotin was the root cause, we investigated methods to balance the signal intensity between the test zones and the control zone. Since high amplicon yield is desirable for LoD, reducing the amount of target DNA was not ideal. Instead, we focused on limiting the initial input of biotin by adjusting the ratio of biotinylated to non-biotinylated primers in the LAMP reaction mix.

Figure 4.12 presents the results of varying the labelled primer ratio (50%, 25%, 10%, and 1% of the total primer concentration) against serial dilutions of Influenza B amplicons (20X to 80X, 40 μ L amplicons added onto each LFS). In this single-plex setup, the test line signals for the 50%, 25%, and 10% ratios showed little variation across different amplicon dilutions. This suggests that even at 10% labelling, the amount of biotinylated amplicon produced is sufficient to saturate the test line capture sites on the strip. However, a clear trend was observed when comparing the lower ratios against the negative control (buffer only). As the percentage of labelled primer decreased from 10% to 1%, the test line signal naturally weakened, but correspondingly, the control line signal became stronger. The 1% ratio resulted in a weaker test signal, which is damaging the sensitivity.

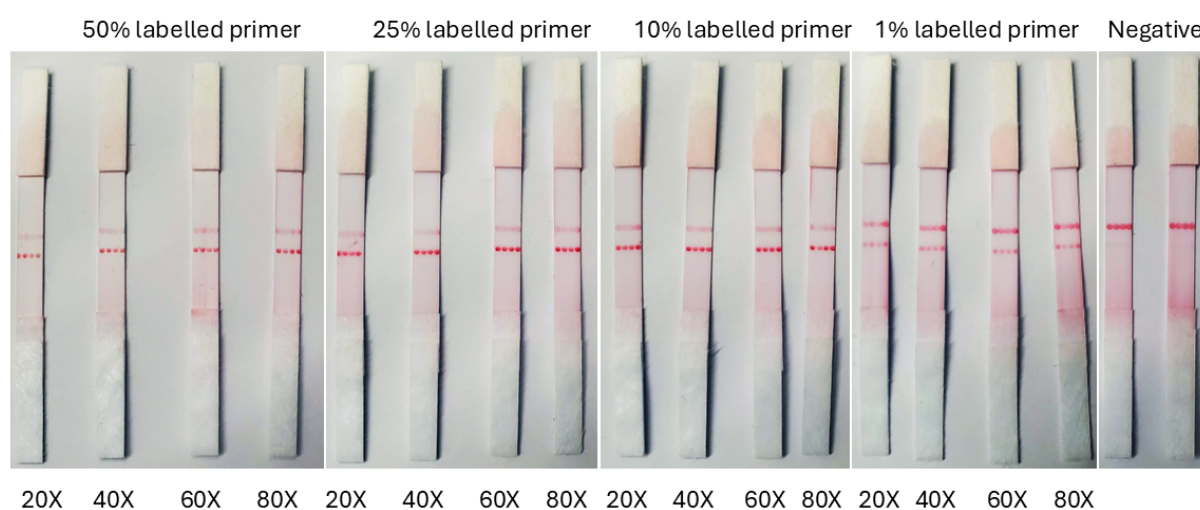


Figure 4.12: **Optimisation of the biotinylated primer ratio to balance test line sensitivity and control line reliability.** Influenza B LAMP reactions were prepared with 50%, 25%, 10%, and 1% labelled primer ratios and tested at various amplicon dilutions (20X-80X). While ratios from 50% to 10% yielded similar saturated test signals in this single-plex format, reducing the ratio to 1% significantly weakened the test signal. Comparison with the negative control (Neg) demonstrates that lower biotin loads (e.g., 10% vs 1%) result in stronger control line signals due to reduced competition for gold nanoparticles. Ratios of 10-25% were selected as the optimal range for multiplex implementation.

Therefore, a balance must be done. The labelled primer ratio must be high enough to generate strong test signals for low-copy targets but low enough to leave sufficient unbound SA-Au for the control line. Based on these results, ratios between 10% and 25% were identified as the optimal range to satisfy both requirements.

This optimisation process is even more critical in a pentaplex format, where five distinct amplicons compete for the same pool of gold conjugate. The configuration requires careful fine-tuning of antibody capture efficiency and amplicon biotinylation levels for each target. Targets with inherently weaker amplification efficiency or antibody affinity were assigned higher labelled primer ratios and placed further upstream on the strip to prioritise their capture. Following extensive empirical testing, the final optimised configuration (as detailed in Section 2.4.4) utilised labelled primer ratios of 25% for Inf A, RSV A, and RSV B, and 10% for Inf B and COVID. This balanced approach ensured robust detection of all five targets while maintaining a consistent and reliable control line signal.

4.1.5 LoD Analysis of Multiplex Lateral Flow Strips

Evaluating the LoD of a multiplex system is complex due to the number of possible target combinations. To simplify this process, RSV B was selected as the representative target for LoD testing. This choice was strategic: the capture dots for RSV B are positioned at the most downstream location of the test area (as shown in Figure 4.7). Therefore, successful detection of RSV B represents the worst-case scenario for reagent transport, serving as a robust indicator of the strip's overall flow and capture efficiency.

The LoD assessment followed a serial dilution protocol similar to that described in Section 4.1.2. The template DNA concentration was adjusted based on its specific base-pair length (details in Section 2.1) to ensure accurate copy number quantification. The dilution series ranged from 10^5 copies/reaction down to a theoretical 1 copy/reaction.

Real-time LAMP amplification was first performed to establish the baseline LoD of the assay itself. The results, recorded on the QuantStudio™ 3 Real-Time PCR System, are presented in Figure 4.13.

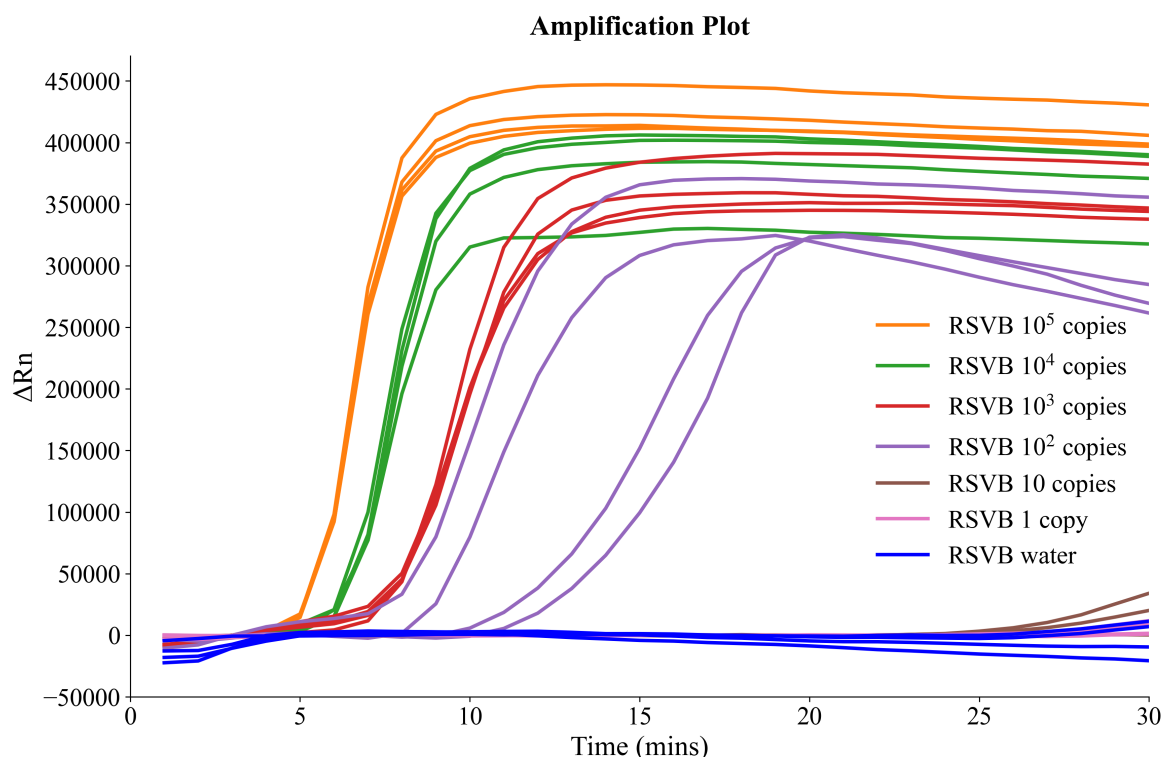


Figure 4.13: **Real-time amplification plots for RSV B serial dilutions.** (Left) Amplification curves for template concentrations ranging from 10^5 to 10^2 copies/reaction, showing consistent positive results. (Right) Amplification traces for 10 copies, 1 copy, and water (NTC), showing no significant amplification. These results establish a reliable detection limit of 100 copies/reaction for the RSV B assay.

The left panel displays the amplification curves for template concentrations from 10^5 to 10^2 copies/reaction. All replicates in this range showed consistent, robust amplification with typical exponential growth kinetics. The right panel shows the results for lower concentrations (10 and 1 copy/reaction). Here, no significant amplification was observed, indicating that the limit of the reaction lies between 100 and 10 copies. Based on these data, the reliable Limit of Detection (LoD) for the RSV B LAMP assay was roughly determined to be 100 copies/reaction.

Subsequently, these same amplicons were tested on the fabricated multiplex lateral flow strips to verify if the paper-based readout maintained this sensitivity. Figure 4.14 illustrates the results. Clear, specific red dots corresponding to the RSV B capture zone were visible for samples containing 10^5 , 10^4 , 10^3 , and 10^2 copies/reaction. Importantly, the control line remained distinct across all tests, confirming that the optimised primer ratios successfully prevented conjugate depletion. At 10 copies/reaction, the signal faded to background levels, matching the negative results seen in the real-time amplification plots. The No Template Control (NTC) showed only the control line, confirming the absence of non-specific binding. This consistency between the

real-time LAMP data and the lateral flow readout confirms that the multiplex strip format introduces no significant loss of sensitivity. The system is capable of reliably detecting downstream targets down to 100 copies/reaction, a limit sufficient for clinical relevance in acute respiratory infections.

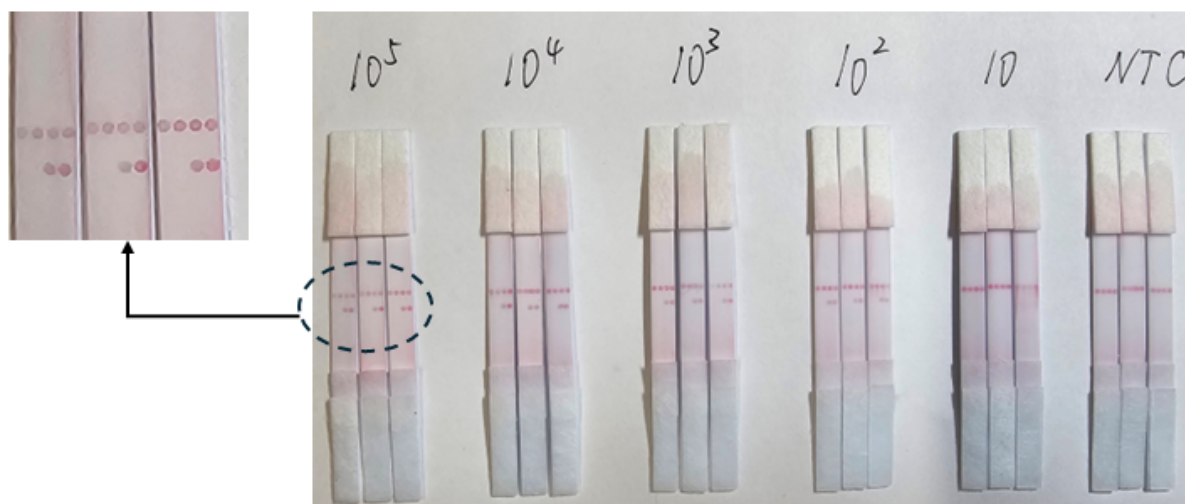


Figure 4.14: **LoD assessment of the multiplex lateral flow strip using RSV B amplicons.** Distinct positive signals at the specific RSV B capture dots are visible for concentrations from 10^5 down to 10^2 copies/reaction. At 10 copies/reaction and in the NTC, only the control line is visible. The detection limit of 100 copies/reaction on the strip matches the LoD of the real-time amplification, validating the performance of the downstream multiplex capture zone.

4.1.6 Shelf-life and Stability of Multiplex Lateral Flow Strips

To determine the shelf-life of the fabricated multiplex lateral flow strips, two batches of strips were stored in a refrigerator at 4 °C. The stability was assessed by running pentaplex (5 targets), triplex (3 targets), and negative control samples to verify signal intensity and background clarity.

Figure 4.15 presents the results after 9 months and 18 months of storage. The strips tested after 9 months demonstrated high stability. The penta-plex and triplex tests showed distinct, specific positive signals for all target dots, comparable in intensity to fresh strips. The negative controls also remained clean, indicating that the blocking agents and immobilised antibodies had not degraded in a manner that would cause non-specific binding. In contrast, the performance of the strips stored for 18 months was significantly worse. As shown in the right panel of Figure 4.15, the results were inconsistent and largely uninterpretable. Many capture dots failed to appear, and

most notably, the control line was frequently absent or very faint. This failure is possibly caused by the degradation of the capture antibody over time. Antibodies immobilised on nitrocellulose are prone to denaturation or desorption during prolonged storage, leading to a loss of binding affinity (Ma *et al.* 2020, Kang *et al.* 2021). Furthermore, the streptavidin-gold conjugate on the conjugate pad may have aggregated or lost activity, preventing successful capture.

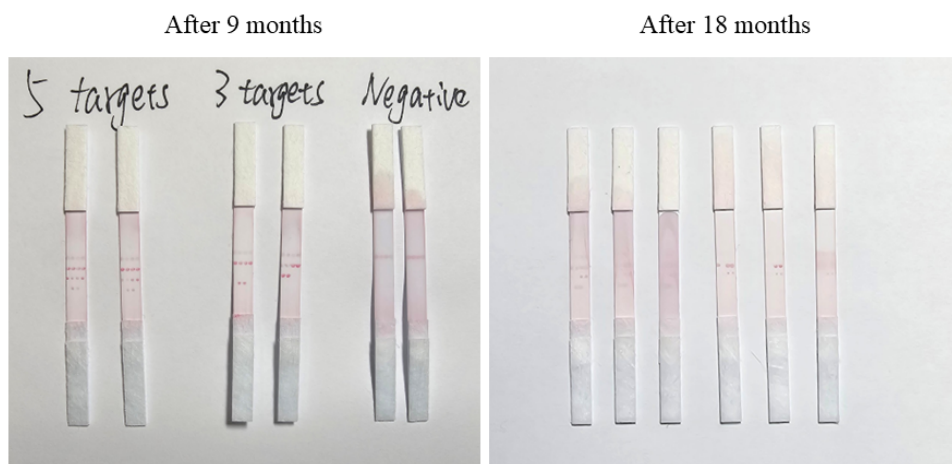


Figure 4.15: **Evaluation of multiplex strip performance after long-term storage at 4 °C.**(Left) After 9 months, the strips maintain high functionality, showing clear specific signals for 5-target and 3-target samples with clean negative controls. (Right) After 18 months, significant degradation is observed, characterised by missing capture dots and absent control lines, indicating loss of reagent activity.

These results suggest that the current fabrication protocol yields strips with a reliable shelf-life of at least 9 months under refrigerated conditions, which is sufficient for typical research and initial field-testing cycles. However, extending stability beyond one year would likely require additional stabilisation agents (for instance, trehalose) in the dispense and conjugate buffers to preserve protein structure.

4.1.7 Feasibility of On-Paper LAMP Amplification

Following the successful optimisation of the lateral flow strip, the physical transport of amplicons from the reaction zone to the detection zone emerged as a critical integration challenge. Integrating nucleic acid amplification directly into a paper matrix offers a streamlined solution for device architecture, potentially allowing the reaction product to wick directly to the detection strip without manual pipetting. However, the porous structure and chemical composition of the

paper can interfere with the reaction kinetics or the optical detection methods used to monitor amplification. Therefore, the first step in developing the on-paper module was to assess whether the LAMP reaction could proceed efficiently within a paper substrate and whether standard fluorescence detection methods remained valid.

To investigate this, a series of experiments was conducted using glass fibre paper. The material is selected due to its high void volume and wide application in commercial lateral flow diagnostics (Koczula *et al.* 2016). Glass fibre sheets were cut into 5×5 mm squares and placed into standard PCR tubes. A 25 μ L LAMP reaction volume (containing Influenza B template for positives) was pipetted directly onto each square. A baseline control group consisting of the standard liquid-phase LAMP assay was run in parallel.

The real-time amplification results are presented in Figure 4.16. Panel (A) displays the baseline liquid reaction, showing clear separation between the positive amplification curves and the flat negative controls. In contrast, Panel (B) shows the results from the paper-based reactions. While amplification signals were generated, a significant rise in fluorescence was also observed in the negative controls containing paper. This unexpected background signal made it difficult to distinguish between true positive and non-specific amplification, suggesting that the glass fibre matrix might scatter light or emit significant fluorescence that worsens the analytical fluorescence performance in the real-time thermocycler (Luongo *et al.* 2023, Shah *et al.* 2017).

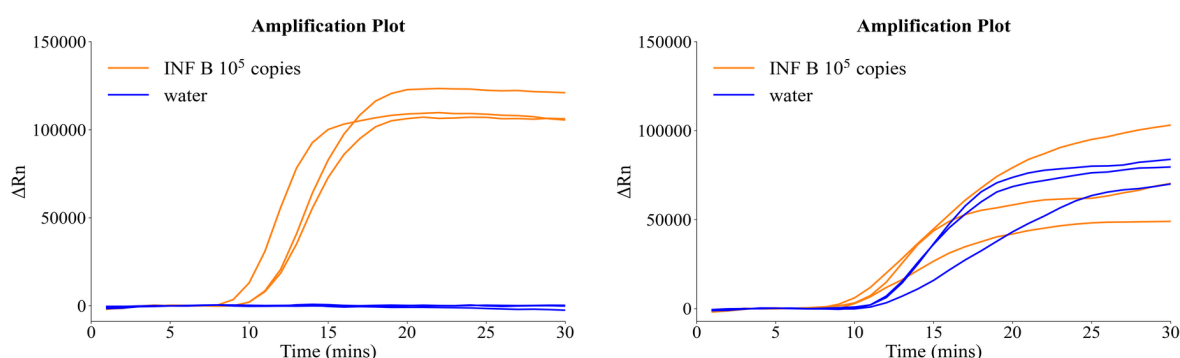


Figure 4.16: **Comparison of real-time LAMP amplification in liquid vs. on paper.**(A) Baseline liquid-phase reaction showing distinct amplification for positives (10^5 copies) and flat lines for negatives (water). (B) Reaction performed on glass fibre squares, showing rising fluorescence signals in both positive and negative samples, indicating significant optical interference or non-specific background generated by the paper matrix.

To further clarify the source of this interference and evaluate alternative readout methods, a comprehensive screening was performed using a fluorescence plate reader. Three distinct heating and containment configurations were evaluated to determine if the container type influenced the readout (Amplification time 30 mins):

1. **qPCR machine:** The paper squares were placed inside standard PCR tubes and subjected to precise thermal cycling (isothermal hold).
2. **Heat Block:** The paper squares were similarly contained in closed PCR tubes but heated via a metal heat block.
3. **Incubator:** To mimic a more integrated device setup without rigid tubes, the reagent-soaked paper squares were wrapped in Parafilm to prevent evaporation and placed inside a temperature-controlled incubator at 65 °C.

The fluorescence intensity readings from these conditions are detailed in Table 4.2. The data revealed that the background noise was not a result of the heating method or the paper itself. Dry paper and paper wetted with water showed negligible fluorescence (readings of 43 and 29, respectively). However, the addition of the LAMP primer mix alone caused a high signal spike (reading of 756), significantly higher than the master mix (reading of 93).

Table 4.2: **Fluorescence intensity readings of paper-based reactions under various conditions.** Excitation: 485/20 nm, Emission: 528/20 nm. The high background caused by labelled primers renders fluorescence monitoring ineffective.

Sample ID	Condition	Fluorescence Intensity
1-3	Paper in qPCR machine (Positive)	156, 147, 201
4-6	Paper in qPCR machine (Negative)	172, 105, 208
7	Heat block 60 min (Positive)	446
8	Heat block 60 min (Negative)	396
9-10	Incubator 60 min (Positive)	454, 459
11	Incubator 60 min (Negative)	430, 443
12	Dry paper only	43
13	Paper + Water	29
14	Paper + Master Mix	93
15	Paper + Primer Mix	756

This indicates that the FITC-labelled primers are the primary source of optical interference. Unlike intercalating dyes that fluorescence only upon binding DNA, the FITC label is constitutively fluorescent. Without a prior washing or spatial separation step, the unreacted primers cannot be removed from the detection zone. Furthermore, the dense, highly scattering nature of

the glass fibre matrix traps and diffuses this baseline emission. Consequently, the constitutive fluorescence from the free primers completely drowns out the target signal, rendering the difference between positive and negative samples statistically insignificant across all three heating methods (e.g., 446 vs. 396 in the heat block).

These findings demonstrate that direct real-time fluorescence monitoring might not be feasible for this on-paper assay due to the high signal-to-noise ratio caused by the labelled primers.

Since the high background fluorescence from labelled primers obscured real-time detection, the next phase of the investigation focused on selecting the optimal paper substrate and validating the reaction. Two types of porous matrices were evaluated: Whatman Grade 1 filter paper (cellulose-based) and Standard Glass Fibre paper. Prior to use, 5×5 mm squares of each material were sterilised via UV irradiation to eliminate potential contaminants.

The real-time amplification curves for these substrates are compared in Figure 4.17. A distinct difference in performance was observed between the materials. The reactions on filter paper (Panel A) exhibited clear separation between positive and negative samples. In contrast, the glass fibre substrate (Panel B) produced chaotic and uninterpretable amplification profiles.

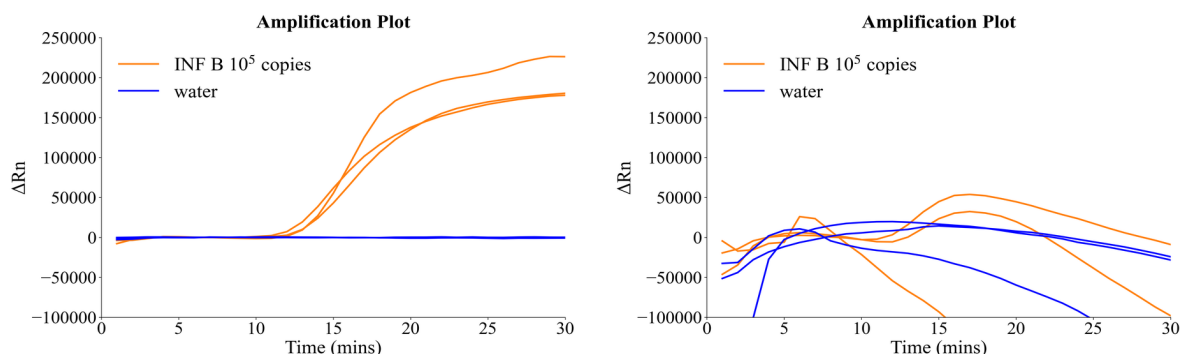


Figure 4.17: **Real-time amplification profiles of LAMP reactions on different paper substrates.** (A) Reaction on cellulose filter paper. (B) Reaction on glass fibre paper. In both cases, the curves are erratic, with significant baseline drift in negative controls, confirming that real-time optical monitoring is unreliable regardless of the substrate type.

Notably, these glass fibre curves differed significantly from those obtained in the previous experiment (Figure 4.16B), where the curves appeared more regular despite the high background. This lack of reproducibility highlights a fundamental flaw in using a qPCR machine for solid substrates: the position and shape of the paper square within the reaction tube are uncon-

trolled. In the curved bottom of a PCR tube, the paper often deforms or adheres to the side walls rather than lying flat. This random positioning alters the optical path length and scattering properties for each replicate, leading to inconsistent fluorescence readings. Consequently, real-time curves cannot be relied upon as a robust method for monitoring on-paper amplification.

To definitively confirm the amplification and assess the physical release of DNA from the matrices, the reaction products were analysed using gel electrophoresis (1% agarose gel). A key modification to the standard protocol was made: the paper squares themselves were inserted directly into the gel wells using forceps, allowing the DNA to migrate out of the porous substrate during the run.

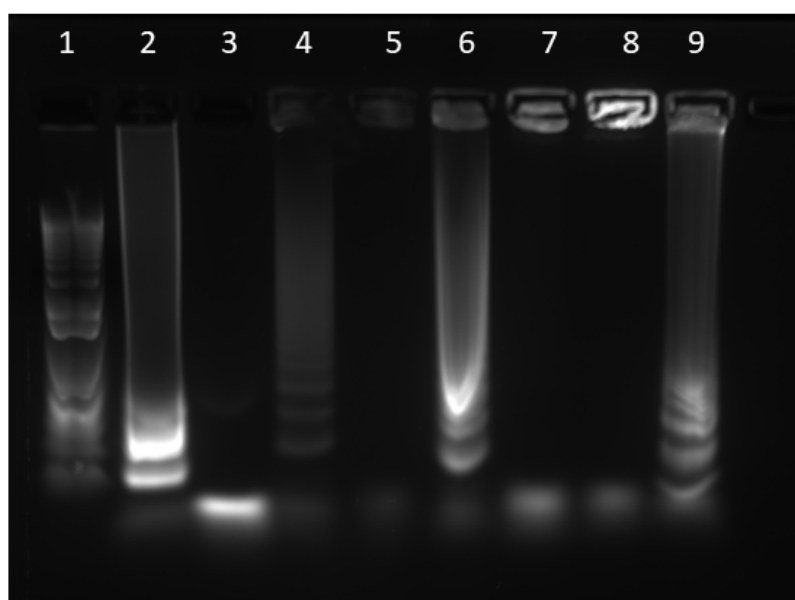


Figure 4.18: **Agarose gel electrophoresis (1%) confirming LAMP amplification on paper substrates.** Paper squares were loaded directly into the wells. **Lane 1:** 1kb Ladder. **Lane 2-3:** Liquid controls (+/-). **Lane 4-5:** Glass fibre (+/-) from qPCR machine. **Lane 6-7:** Filter paper (+/-) from qPCR machine. **Lane 8:** Glass fibre from incubator. **Lane 9:** Filter paper from incubator. Note the strong release of DNA from filter paper (Lanes 6, 9) compared to the retention of DNA in the glass fibre wells (Lane 8), attributed to electrostatic trapping.

The electrophoretic results (Figure 4.18) provided the clarity that the optical methods lacked:

- **Filter Paper (Lanes 6, 7, 9):** The filter paper samples showed distinct, bright bands for the positive reactions (Lanes 6 and 9) and a clean, empty lane for the negative control (Lane 7). This confirms that the LAMP reaction proceeds efficiently within the cellulose matrix and, crucially, that the DNA amplicons are easily released into the gel.

- Glass Fibre (Lanes 4, 5, 8): The glass fibre results were problematic. While some amplification was visible (Lane 4), the DNA migration was slightly retarded. This was most evident in the incubator-heated sample (Lane 8), where the DNA appeared stuck within the well, failing to migrate into the gel.

The poor migration observed with glass fibre is attributed to electrostatic entrapment. DNA is negatively charged, whereas glass fibres often carry surface positive charges or specific oxide groups that bind nucleic acids (Zou *et al.* 2017). This interaction effectively traps the amplicons. In contrast, cellulose-based filter paper is relatively inert and allows for efficient DNA elution, a property essential for downstream transport in a lateral flow device.

An artifact was noted in the gel image: the leading edge of the DNA bands appeared uneven or wavy across the paper-containing lanes. This distortion is likely caused by the physical presence of the paper square disturbing the local electric field, or by the diffusion of high-viscosity buffer components from the paper affecting the migration front.

Table 4.3: **End-point fluorescence intensity of reactions on different paper substrates.** The lack of correlation between positive/negative status and fluorescence intensity confirms that optical monitoring is unreliable for this setup.

Sample ID	Condition	Fluorescence Intensity
1-2	Glass fibre – positive (qPCR)	426, 440
3-4	Glass fibre – negative (qPCR)	660, 556
5-6	Filter paper – positive (qPCR)	1506, 1014
7-8	Filter paper – negative (qPCR)	1513, 1401
9	Glass fibre – positive (incubator)	873, 947
10	Filter paper – positive (incubator)	1601, 1557

Finally, the end-point fluorescence intensity measured by a plate reader (Table 4.3) confirmed the variability seen in the qPCR plots. The negative control readings for filter paper (1513, 1401) were unpredictably high, often exceeding the positive readings (1506, 1014). This difference reinforced the conclusion that the fluorescence intensity on paper is too variable to serve as an amplification indicator.

Based on the superior DNA release properties demonstrated by electrophoresis and the consistent performance despite optical limitations, Whatman Grade 1 filter paper was selected as the optimal substrate for the on-paper amplification module.

4.1.8 Detection of On-Paper Amplicons via Lateral Flow Strips

Gel electrophoresis definitively confirmed that the LAMP reaction occurs efficiently on filter paper. The next critical step was to establish whether these on-paper amplicons could be successfully transferred to and detected by the lateral flow strip, completing the integrated workflow.

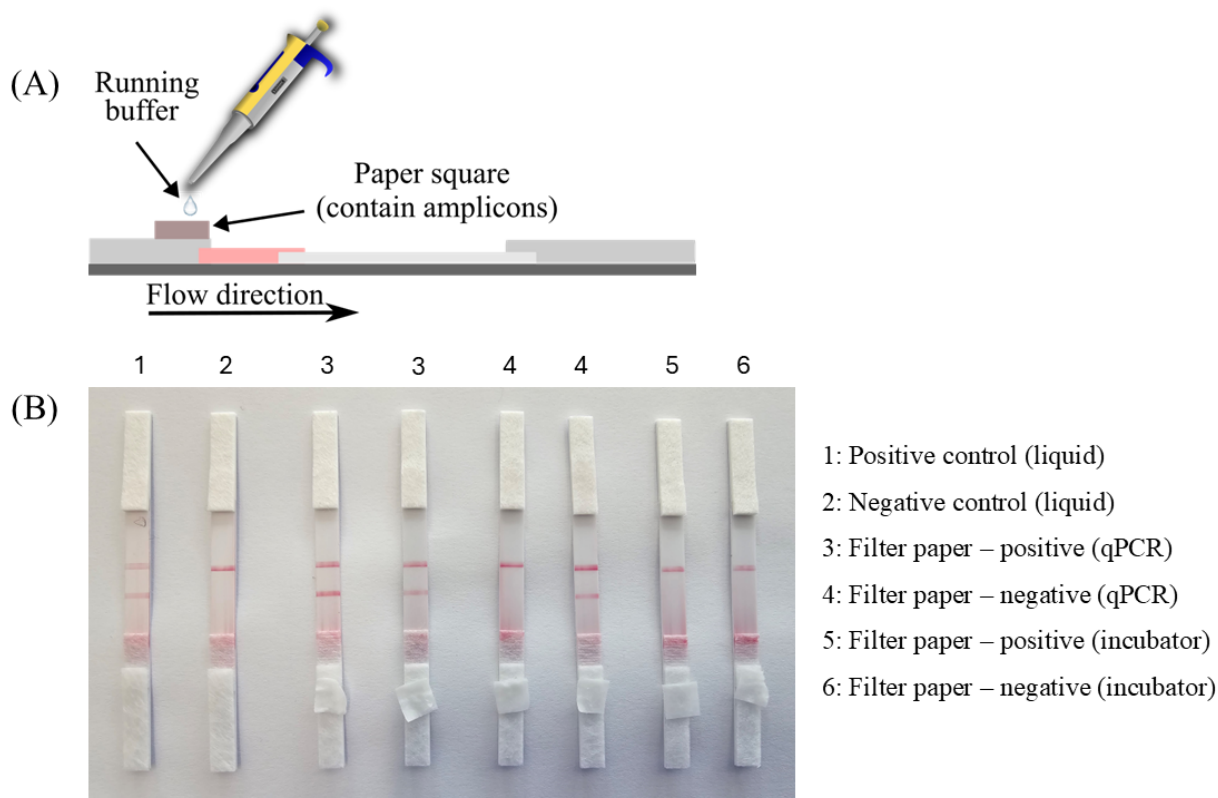


Figure 4.19: **Evaluation of the direct wicking method for on-paper amplicon detection.** (A) Schematic illustrating the physical transfer setup: the paper square containing the completed LAMP reaction is placed flat onto the LFS sample pad. Running buffer is pipetted directly onto the square to elute the amplicons and drive them into the strip via capillary action. (B) Photographic results of the LFS assays. Samples heated in the qPCR machine show inconsistent amplicon release, yielding both weak positives and a false negative. Samples heated in the incubator show no signal, suggesting failure of the buffer to passively elute the large DNA concatemers from the cellulose fibres.

For this proof-of-concept, Influenza B served as the target, employing a standard single-test-line LFS configuration. The initial attempt focused on the simplest possible transfer method: “direct wicking”. To execute this, the cellulose paper squares were physically removed from their reaction vessels (either PCR tubes from the thermocycler or Parafilm wraps from the incubator) with forceps and placed flat against the absorbent sample pad of the LFS. The conceptual mech-

anism relies on vertical elution: a 40 μL volume of running buffer is pipetted directly onto the top surface of the paper square. The buffer is intended to flush through the cellulose matrix, elute the entangled LAMP amplicons, and carry them downward into the sample pad to initiate horizontal capillary flow across the nitrocellulose membrane.

The results, presented in Figure 4.19, were inconsistent. The papers heated in the qPCR machine yielded a visible positive signal, but a false negative was also observed, indicating unreliability. More significantly, the samples heated in the incubator failed to generate any test line signal. Given that the gel electrophoresis results in the previous section had already confirmed successful amplification under these incubator conditions, the lack of a signal on the LFS pointed to a transport issue rather than a reaction failure. It appeared that simply adding a buffer was insufficient to passively release enough DNA amplicons from the cellulose matrix from the incubator samples.

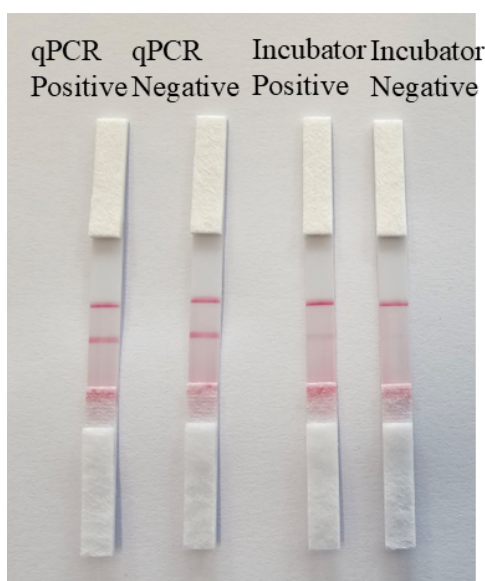


Figure 4.20: **LFS results following active elution of amplicons from the paper squares using TE buffer and mechanical squeezing.** The incubator samples now show correct positive signals, confirming that the previous failure was due to inefficient DNA release. The qPCR samples show signals but also exhibit false positives.

To verify this hypothesis, an active elution step was introduced to facilitate the release of DNA from the substrate. The amplified paper squares were transferred into Eppendorf tubes containing 100 μL of 1X TE buffer (pH 8.0). After several minutes of incubation, the paper was mechanically squeezed using a pipette tip to force the liquid out of the fibres. This resulting eluant was then pipetted onto the strip. As shown in Figure 4.20, the addition of the elution step

yielded immediate improvements. The samples processed in the incubator began to show correct positive signals, indicating that the amplicons were present but had previously been trapped in the paper. However, the signals remained relatively weak. The eluant from the qPCR machine papers continued to yield signals but also showed false positives.

While the elution experiments confirmed that amplicons could be generated on and released from the paper, the heating methods used (PCR tubes, Parafilm wraps) did not present correct and strong signals, and reflecting the real situation of the final integrated device.

To simulate the conditions of the final integrated device, the on-paper reaction was transitioned to a sealed PMMA chamber, fabricated as described in Section 2.2.3. This prototype mimics the geometry and thermal mass of the final microfluidic chip. Four sets of positive and negative replicates were prepared on filter paper, loaded into the chambers, and incubated for the standard 30 minutes at 65 °C.

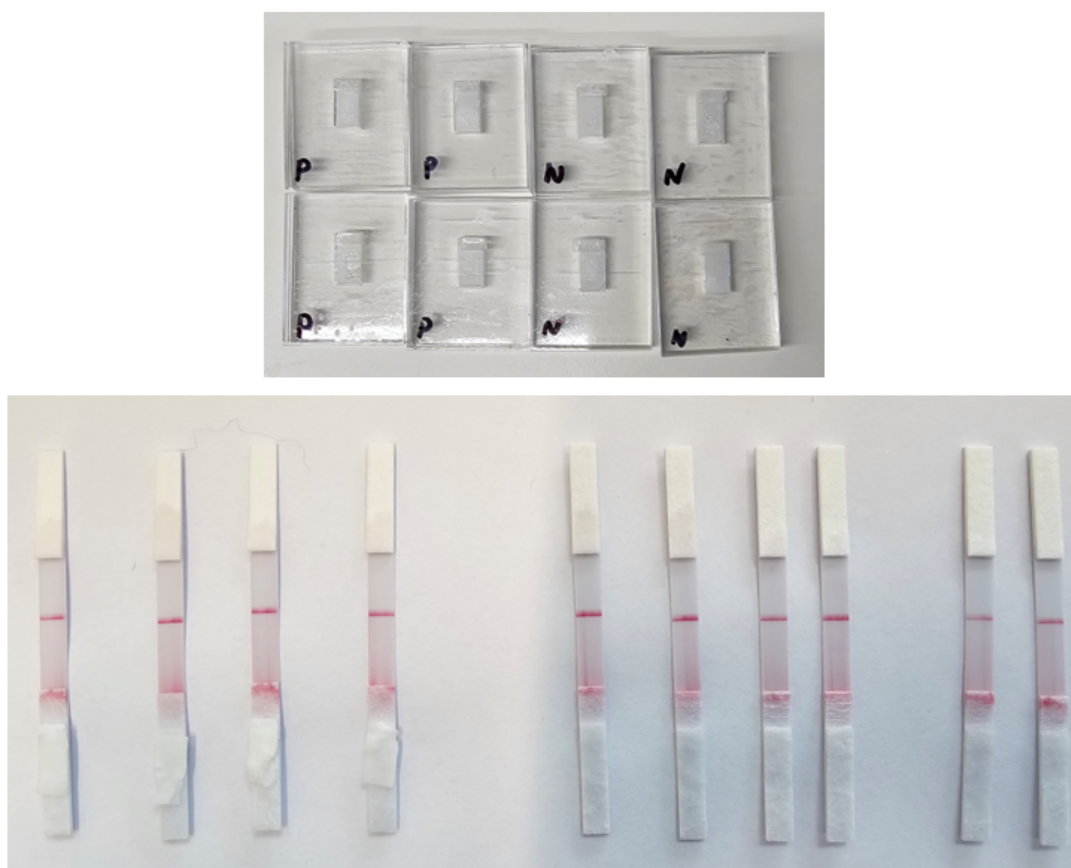


Figure 4.21: **Initial testing of on-paper LAMP within prototype PMMA chambers.** (Top) Photograph of the assembled PMMA chambers loaded with paper squares. (Bottom) LFS results after a standard 30-minute incubation. All tests, including positives, show negative results, indicating insufficient amplification.

The initial results, shown in Figure 4.21, were discouraging. Regardless of whether the paper was applied directly to the strip or subjected to an elution step, every test yielded a negative result. Rather than a biochemical failure, we hypothesised that relying on a standard laboratory incubator for thermal actuation was the root cause. Unlike direct conductive heating (e.g., using an aluminium heat block), an incubator relies on natural air convection. According to classical heat transfer principles (Incropera *et al.* 2011), the natural convective heat transfer coefficient (h) of air is exceptionally low, typically ranging from 2 to 25 $\text{W}/(\text{m}^2 \cdot \text{K})$. In contrast, direct solid-to-solid thermal contact conductance (e.g., aluminium to plastic) easily exceeds 1,000 $\text{W}/(\text{m}^2 \cdot \text{K})$. Furthermore, air possesses an inherently low thermal conductivity ($k \approx 0.026 \text{ W}/\text{m} \cdot \text{K}$, compared to aluminum's $k \approx 237 \text{ W}/\text{m} \cdot \text{K}$) and a volumetric heat capacity ($\sim 1.16 \times 10^3 \text{ J}/\text{m}^3 \cdot \text{K}$) that is roughly 1,500 times lower than that of solid PMMA ($\sim 1.75 \times 10^6 \text{ J}/\text{m}^3 \cdot \text{K}$). Consequently, the heat transfer from the warm air to the solid PMMA cassette is highly inefficient. This large thermal resistance was likely responsible for the delayed ramp rate, leaving the reaction paper with insufficient time at the required amplification temperature.

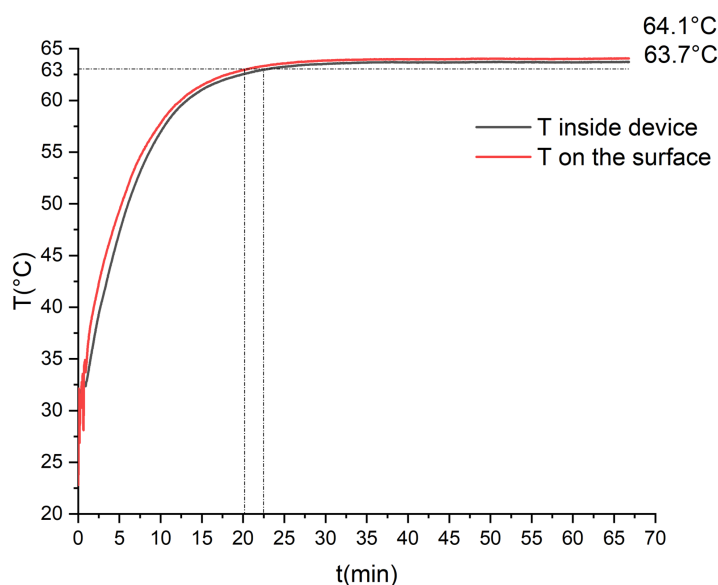


Figure 4.22: **Thermal monitoring of the PMMA chamber inside a 65 °C incubator.** While the internal chamber eventually reaches the target temperature range, the ramp rate is severely delayed due to the inefficient convective heat transfer of the incubator air. It takes nearly 20 minutes to reach a stable plateau, indicating that a 30-minute total reaction time only provides a fraction of the necessary thermal window for the enzyme.

To investigate this thermal dynamic, a thermocouple was embedded inside and attached to the surface of the chamber to monitor the internal temperature profile during incubation. The results, presented in Figure 4.22, confirmed our hypothesis. While the chamber eventually reaches the optimal LAMP temperature range (60–65 °C), the convective heating mechanism causes a significant lag in the ramp rate. It took over 10 minutes for the internal temperature to merely reach 60 °C, and nearly 20 minutes to finally stabilise at the plateau. Consequently, the standard 30-minute protocol provided only a very brief window for effective enzymatic activity. Given that paper-based reactions often require longer times due to diffusion constraints, the 30-minute incubation was obviously insufficient.

Based on this thermal data, the total reaction time was extended to 50 minutes to ensure the paper reached and maintained the target temperature for an adequate duration. The results of this extended run are shown in Figure 4.23. The outcome completely reversed: every sample, including the negative controls, yielded a strong positive signal. This indicated severe unspecific amplification, which is not surprising. LAMP is known to be extremely sensitive and prone to cross-contamination (Naik *et al.* 2019), which is a challenge frequently encountered throughout this study.

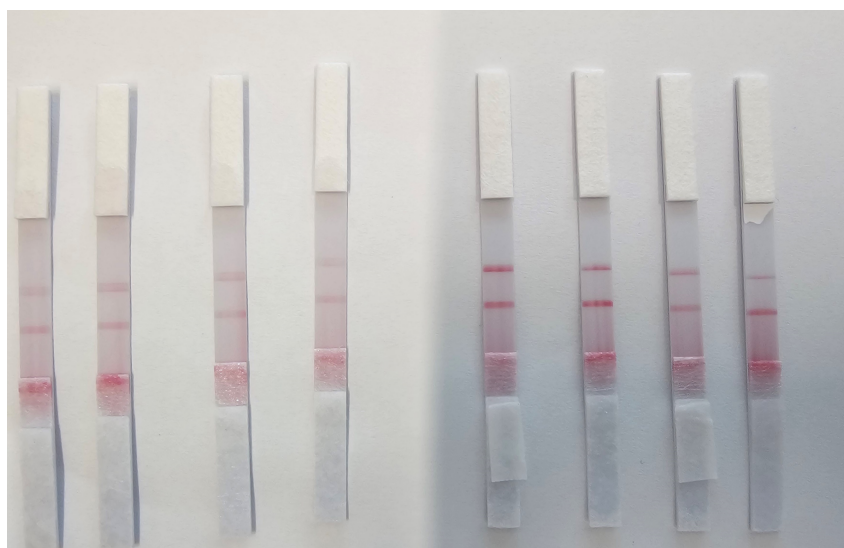


Figure 4.23: **LFS results after extending the PMMA chamber incubation time to 50 minutes.** All samples, including negatives, show strong positive signals. This indicates severe, unexpected amplification might be caused by unclean prototype chambers.

To address this, strict cleaning procedures were implemented. The PMMA chambers were subjected to the decontamination protocol detailed in Section 2.7.2, and fresh filter paper strips were UV-sterilised immediately before loading. A final validation experiment was conducted with two groups incubated for 50 minutes: Group 1 contained reagents only directly in the chamber (no paper) to ensure the chamber material itself was not causing reactions, and Group 2 contained reagents absorbed onto the paper strips.

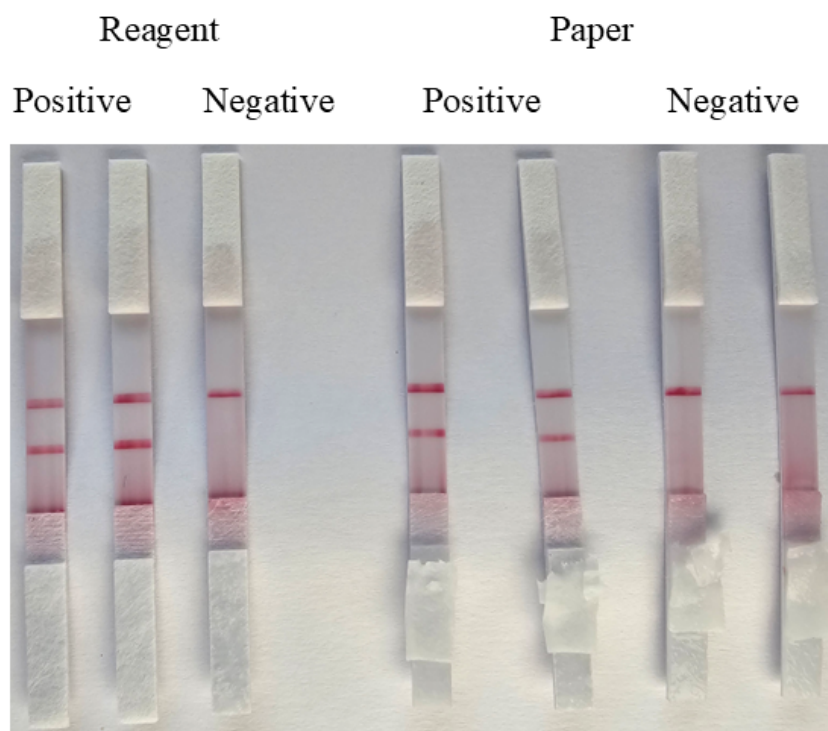


Figure 4.24: **Final validation of on-paper LAMP in PMMA chambers following rigorous cleaning and UV sterilisation protocols (50 min incubation).** (Left) Reagents only (no paper) show correct negative results. (Right) Reagents on paper show distinct positive signals and clean negatives. Amplicons were transferred via direct wicking without an elution step, indicating effective on-chip transfer and integration.

The final, optimised results are presented in Figure 4.24. Following rigorous cleaning and extended incubation, the system performed correctly. Negative controls in both groups remained clean. Most importantly, the positive paper samples generated strong, clear test line signals via direct wicking onto the LFS sample pad, without requiring a separate elution step. This conclusively demonstrated that successful amplification and subsequent passive transfer to the detection zone can be achieved within the PMMA device environment.

4.2 Discussion

4.2.1 Resolution of Spatial Interference via Micro-Dot Geometry

The primary challenge addressed in this chapter was the mutual weakening of signals observed during the development of the penta-plex assay. As detailed in the preliminary testing (Section 4.1.3), the initial line-format prototypes exhibited a depletion effect, where the binding of high-concentration upstream targets significantly suppressed the signal of downstream targets. This finding aligns with the limitations of sequential flow described in the literature (Bartosh, Sotnikov, Zherdev, *et al.* 2023). Still, the severity of the signal loss observed here highlights a specific vulnerability when applying standard strip architecture to high-yield LAMP amplicons.

By transitioning to the micro-dot array, this work successfully extends the multiplexing detection to five targets. The experimental data from the penta-plex validation (Section 4.1.5) demonstrates that the downstream RSV B dots maintained a LoD of 100 copies/reaction (equivalent to 20 copies/ μL), same as the single-plex control. This level of sensitivity is highly competitive for multiplexed lateral flow assays. Expanding target numbers on a single porous membrane often introduces steric hindrance and reagent depletion, which typically compromises analytical sensitivity. For instance, a recently developed multiplex LFA targeting three distinct human papillomavirus (HPV) genotypes reported LoDs ranging from 10 to 100 copies/reaction (Barra *et al.* 2024). Similarly, another recent multiplex lateral flow platform designed for the simultaneous detection of three veterinary respiratory viruses (BVDV-1, BEFV, and BRSV) reported LoDs of 2.62×10^1 , 2.42×10^1 , and 2.56×10^1 copies/ μL , respectively (Y. Song *et al.* 2025). Crucially, in acute respiratory infections such as RSV or COVID-19, the viral load in clinical nasopharyngeal swabs typically ranges from 10^4 to 10^8 copies/mL (10 to 100,000 copies/ μL) during the symptomatic phase (Wölfel *et al.* 2020). Therefore, an achieved LoD of 20 copies/ μL easily captures clinically infectious patients, firmly validating the application relevance of this device.

The success of this multiplexing approach is rooted in its geometry. Unlike the continuous barrier of a test line, which forces the entire sample volume to pass through the first capture zone, the isolated dots allow a significant fraction of the fluid stream that carries unbound colloidal gold and downstream amplicons to flow around the initial capture sites. This flow diversion mechanism ensures that the local concentration of reporter particles remains sufficient for the most distal capture zones, enabling the simultaneous detection of five targets with uniform sensitivity.

Furthermore, the results present a critical economic and efficiency advantage of the dot configuration over alternative multiplexing strategies. While other high-sensitivity labels, such as quantum dots or SERS nanotags, could theoretically overcome signal limitations through higher luminosity, they require complex external excitation equipment that contradicts the point-of-care mandate of this project (Sloan-Dennison *et al.* 2022, Foubert *et al.* 2017). The micro-dot approach achieved the required multiplexing capability using standard colloidal gold, maintaining instrument-free readability.

In addition, this geometry also optimised reagent consumption. During the fabrication, we found that the dot format required approximately 3 to 4 times less capture antibody volume than the equivalent line format to achieve comparable signal intensity. This reduction is significant not only for cost-effectiveness but also for assay development; it permitted the use of high-affinity, expensive monoclonal antibodies for all five targets without exceeding the cost constraints of a disposable device. Thus, the micro-dot array had a functional necessity for accommodating the high reagent demand of a penta-plex nucleic acid assay on a compact lateral flow strip.

While the micro-dot geometry effectively resolves spatial interference and reduces reagent consumption, it inherently introduces new complexities for scalable manufacturing. As identified during prototyping (Section 4.1.3), the discrete nature of the dots makes the strips highly vulnerable to lateral misalignment during the cutting phase, necessitating a lower-throughput “cut-then-print” workaround for laboratory fabrication. However, for future translation to high-throughput industrial manufacturing, this limitation can be readily overcome using established Roll-to-Roll web processing technologies. By printing the dot arrays onto continuous rolls of nitrocellulose, accompanied by optical fiducial markers, industrial machine-vision systems can actively guide precision rotary slitting knives to cut perfectly between the dot columns. This ensures that the highly efficient “print-then-cut” paradigm can be safely utilised at scale, preserving the diagnostic benefits of the dot array without sacrificing manufacturing throughput.

4.2.2 Competitive Binding Dynamics in High-Yield LAMP Amplicons

A recurring challenge encountered during the optimisation of the multiplex strip was the conjugate depletion. In this scenario, the successful amplification of the target accidentally led to the disappearance of the control line, rendering the test invalid. This phenomenon is distinct from the flow-based interference discussed in the previous section. Instead, it is a consequence of the intense competitive binding dynamics in the Biotin-Streptavidin-Gold reporting system when exposed to the high product yield generated by LAMP.

Unlike PCR, which is a discrete cycling process that typically reaches a plateau of DNA yield determined by cycle count, LAMP is a continuous, high-efficiency isothermal reaction. It often generates amplicon concentrations orders of magnitude higher than PCR, with DNA amplified $10^9 - 10^{10}$ in 15-60 min (Parida *et al.* 2008). In the context of a lateral flow assay, this efficiency presents a specific challenge. The reaction mixture applied to the strip contains not only a high concentration of dual-labelled amplicons but also a significant molar excess of residual biotinylated primers that did not participate in the reaction (Agarwal *et al.* 2022).

The experimental investigation in Section 4.1.4 confirmed that excess biotin is the root cause of the signal loss. As quantitatively established in Section 4.1.4, the system operates under a severe imbalance. A standard LAMP reaction introduces 5.0 to 10.0 pmol of biotinylated primers. In contrast, the theoretical maximum binding capacity of the 6 μ L SA-Au conjugate pad is highly restricted, offering only 1.2 to 2.0 pmol of accessible biotin-binding sites. Thus, even under the most optimistic geometrical estimates, the initial primer input alone represents a 2.5- to 5-fold excess over the reporter capacity.

Upon release from the conjugate pad, the finite population of Streptavidin-Gold nanoparticles interacts immediately with the biotinylated amplicons in the solution. Due to the exceptionally high affinity of the streptavidin-biotin interaction, the binding sites on the streptavidin become rapidly saturated before the fluid front has passed through the test zone. When this saturated complex reaches the control line, which typically relies on capturing unoccupied streptavidin on the gold surface, there are no free binding sites remaining. Consequently, the gold nanoparticles pass over the control line without being captured, resulting in a false invalid result. This mechanism is similar to the hook effect commonly observed in immunoassays (Ross *et al.* 2020), but it is driven here by the amplification efficiency rather than antigen excess.

More importantly, this work demonstrated that the resolution to this imbalance lies in manipulating the label input rather than increasing the gold concentration, which would inevitably increase background noise and manufacturing costs. By adjusting the labelled primer ratio, a functional equilibrium was established. The experimental data indicated that reducing the biotinylated primer fraction to 10–25% of the total primer concentration did not compromise the sensitivity of the test dots. This suggests that the binding capacity of capture dots is the limiting factor in signal generation. Providing excess biotin beyond what the test dots can capture contributes nothing to the diagnostic signal and only suppresses the control line. The dilution of amplicons is also reported as the solution to alleviate the hook effect as well as signal loss (Agarwal *et al.* 2022). However, it is not feasible in our project because we cannot finish such dilution with a running buffer in an integrated device, given that the test zone signal is still significant after 100-fold dilution.

This finding revealed a critical design distinction between PCR-based and LAMP-based lateral flow assays. Standard LFS design rules often assume the abundance of capture reagents relative to the target. However, for high-yield isothermal amplification, reagent components must be strictly managed to prevent the reporter system from being overwhelmed by the amplification products.

4.2.3 Storage Conditions and Thermal Stability Challenges

As demonstrated in Section 4.1.6, the shelf-life evaluation confirmed that the multiplex lateral flow strips maintained robust signal capture and consistent flow dynamics when stored at 4 °C. While ideal POC diagnostics must ultimately be formulated for prolonged room-temperature storage or elevated temperatures in tropical climates, 4 °C was deliberately selected as the primary benchmark for evaluating this proof-of-concept prototype. Because the novel multiplex dot-array relies on the precise spatial and structural integrity of closely printed capture antibodies, establishing the fundamental biochemical viability of the array under optimal cold-chain conditions was a mandatory initial step to validate the printing mechanics.

Accelerated ageing studies, such as storage at 37 °C or 45 °C to simulate long-term ambient degradation, are routinely employed in the commercial validation of in vitro diagnostic devices (World Health Organization 2019). However, achieving stability under these conditions presents a significant biochemical challenge for antibody-based lateral flow assays. Antibodies immobilised directly onto nitrocellulose membranes are inherently susceptible to thermal

stress, with elevated temperatures promoting irreversible protein denaturation and a pronounced loss of antigen-binding activity (Ma *et al.* 2020). To mitigate this degradation, diagnostic developers typically undertake a dedicated optimisation of protective excipients. Non-reducing disaccharides, most notably trehalose and sucrose, are widely adopted due to their ability to stabilise proteins during drying and storage. These sugars act via preferential hydration, effectively replacing water molecules at the protein surface and forming a rigid, glass-like matrix upon desiccation that preserves the native antibody conformation and binding functionality during prolonged storage at elevated temperatures (Jonsson *et al.* 2024).

As the primary scope of this study was to pioneer and validate the spatial resolution and conjugate balancing mechanics of the multiplex architecture, standard 4 °C storage was utilised. This deliberately decoupled the physical validation of the dot-array from the extensive chemical formulation required for extreme thermal stabilisation. The incorporation of sugar-based lyoprotectants to facilitate ambient storage and accelerated aging validation remains a critical subsequent step for the future commercial translation of this multiplex platform.

4.2.4 On Paper Amplification and Detection

The transition of the LAMP reaction from a liquid phase to a porous paper matrix introduces severe optical and physicochemical interferences. While real-time fluorescence monitoring is the gold standard in liquid tubes, porous substrates like glass fibre and cellulose create a highly scattering optical environment. The dense fibrous networks increase both the optical path length and the scattering cross-section for excitation light (Ulep *et al.* 2018). Furthermore, the constitutive fluorescence of unpurified labels (such as FITC) becomes concentrated on the high-surface-area fibres, creating a baseline intensity that saturates the detector's dynamic range and completely obscures specific amplification signals (Shah *et al.* 2017). Consequently, standard optical monitoring is fundamentally incompatible with opaque, porous substrates without specialised surface-reading instrumentation.

The fundamental reason for embedding the LAMP reaction within a porous matrix is to exploit the material's extensive void volumes and interstitial capillary networks. These microscopic vacancies serve as localised micro-reactors that naturally sequester the fluid, vastly simplifying sample handling and reagent storage for POC applications (Anushka *et al.* 2022). However, this structural advantage introduces the distinct physical challenge of amplicon retrieval: while the matrix must retain the liquid during amplification, it must concurrently permit the rapid

elution of the DNA amplicons for downstream lateral flow detection. Although glass fibre is practically ubiquitous in diagnostic pads due to its wet strength, it exhibits a strong tendency to trap high-molecular-weight DNA. Depending on manufacturing treatments, glass matrices often possess surface oxide groups or residual cationic charges that electrostatically bind the highly negatively charged nucleic acids (Vandeventer *et al.* 2013, B. Shi *et al.* 2015). In contrast, the hydroxyl groups on Whatman Grade 1 cellulose fibres interact with molecules primarily through weaker hydrogen bonding rather than strong ionic attraction (Zandieh *et al.* 2025, R. Shi *et al.* 2018). This inherently lower electrostatic affinity prevents the large, branched LAMP amplicons from becoming permanently bound, allowing efficient passive release into the running buffer for downstream lateral flow detection.

Finally, housing this paper-based reaction within a polymer prototype encountered a critical thermodynamic bottleneck. The severe thermal lag observed during testing was not solely a consequence of PMMA's inherent thermal mass (X. Liu *et al.* 2024), but rather a fundamental limitation of the heating modality. Standard laboratory incubators rely on ambient convective heating. Because air has exceptionally low thermal conductivity and volumetric heat capacity, convective heat transfer from warm air across the solid polymer cassette is highly inefficient. This inefficient transfer leaves the internal reaction mix below the necessary 60–65 °C threshold for Bst polymerase activity for a substantial portion of the incubation period. Therefore, for effective POC device translation, relying on passive ambient air heating is thermodynamically unviable. Future device architecture must integrate highly localised, direct-contact heating elements (such as printed PTC heaters) to enforce rapid conductive heat transfer, thereby bypassing the insulative boundary layer of air entirely.

4.3 Contribution to Knowledge

This chapter details the development and validation of the multiplexed lateral flow strips and the on-paper amplification. Through the systematic optimisation of the micro-dot array and the investigation of the physicochemical interactions within the device, this work makes several contributions to the field of paper-based diagnostics:

Our empirical comparison revealed that conventional multi-line formats suffer from severe signal depletion for downstream targets (the hook effect) when challenged with high-concentration amplicon mixtures. By characterising the micro-dot geometry, we provided a proven solution that utilises flow diversion to preserve reagent availability across the entire detection field. This establishes a scalable design blueprint for future lateral flow assays requiring ≥ 5 targets on a single compact strip without sacrificing sensitivity.

We investigated the phenomenon of control line disappearance, a common but often undiagnosed failure mode in LAMP-based lateral flow assays. We determined that the excess of biotinylated primers generated by efficient isothermal amplification saturates the streptavidin-gold reporter system. By establishing an optimised labelled primer ratio window of 10–25%, we contributed a robust, cost-effective method to balance signal intensity between test and control zones, and provide a critical protocol adjustment for any researcher coupling high-yield amplification with lateral flow detection.

Through comparative analysis of amplification on glass fibre versus cellulose filter paper, we demonstrated that standard glass fibre matrices actively retain DNA amplicons through electrostatic interactions, rendering them unsuitable for downstream wicking. We validated that cellulose filter paper is a better reaction matrix that supports efficient amplification and passive release of amplicons. Furthermore, our thermal profiling of the PMMA micro-chamber proved slower and dissipated heat transfer, establishing the necessity for extended reaction times to compensate for heat transfer lag in point-of-care device prototypes.

Development and Evaluation of the Integrated Microfluidic Platform

The preceding chapters established the core functional components of the diagnostic platform: the self-regulating paper-based heater (Chapter 3) and the optimised multiplexed lateral flow assay (Chapter 4). However, realising a truly portable integrated device requires the integration of these elements with autonomous fluidic control. Conventional microfluidic systems often rely on external syringe pumps or complex electromechanical valves to sequence biochemical steps, requirements that undermine the advantages of portability and low-cost fabrication (X. Zhang *et al.* 2020).

To address these challenges, this chapter presents the development of a integrated, thermally actuated microfluidic platform. By exploiting the principle of thermal coupling, the device exploits the heat required for the LAMP reaction to trigger phase-change paraffin valves, thereby synchronising fluid transport with the amplification process without the need for active electronic actuators.

This chapter details the design, fabrication, and iterative optimisation of the integrated device architecture. The experimental workflow begins with the characterisation of the paraffin-based valves (Sections 5.1.1–5.1.2), specifically establishing the linear geometric dependence of actuation timing to enable programmable fluidic control. Subsequently, the integration of the paper-based reaction strip into the PMMA housing is evaluated. A significant portion of this investigation is dedicated to resolving critical failure modes, including “interfacial bypassing” caused by the enhanced wetting properties of the LAMP reagents (Section 5.1.4) and thermodynamic instability resulting from evaporation in open-chamber geometries.

Following the structural evolution from the “sandwiched” to the “connected” and finally the “elongated” chamber designs (Section 5.1.5), the optimisation of reaction volumes is detailed to buffer against chemical inhibition. Finally, the fully optimised device is validated through a comprehensive LoD analysis (Section 5.1.6), demonstrating the restoration of analytical performance comparable to the benchtop assay and confirming the successful synthesis of thermal, fluidic, and biochemical functions into a unified diagnostic platform.

5.1 Results

5.1.1 Characterisation of Wax Gate Actuation Mechanisms

The full integration of the LAMP assay with lateral flow strip detection requires a reliable fluidic control mechanism to separate the amplification phase from the detection phase. Without a valve, the reaction mixture would wick prematurely into the detection zone, leading to assay failure. To achieve automated, instrument-free operation, thermally actuated wax gates were investigated as passive valves that can be triggered by the heat of the reaction itself or a secondary thermal stimulus.

Two types of wax gates were fabricated using Paraffin-based wax and Candelilla wax, following the protocols detailed in Section 2.6.3. A critical step in the fabrication process was the thermal curing of the wax after printing or deposition. As demonstrated in Figure 5.1(A), the uncured wax gate (left) failed to stop the fluid flow, allowing dye solution to pass through immediately. This failure occurred because the printed wax resides only on the surface of the paper. In contrast, the cured strip (right) successfully blocked fluid transport. The curing process melted the wax, allowing it to penetrate deep into the cellulose fiber matrix to form a complete hydrophobic barrier across the entire cross-section of the paper (Carrilho *et al.* 2009).

To select the optimal material for the device, the thermal response of both wax types was evaluated. Strips containing 2 mm wide gates were placed on a hot plate set to temperatures ranging from room temperature (RT) to 85 °C. Due to rapid evaporation in the open environment, the observation window for each temperature point was limited to 1 minute. The results are presented in Figure 5.1(B). Below 65 °C, both wax gates remained solid and effectively blocked fluid flow. At 65 °C (the target temperature for LAMP), distinct behaviours emerged. The Candelilla wax gate (left, translucent strip) showed early signs of liquid penetration, whereas the Paraffin-based

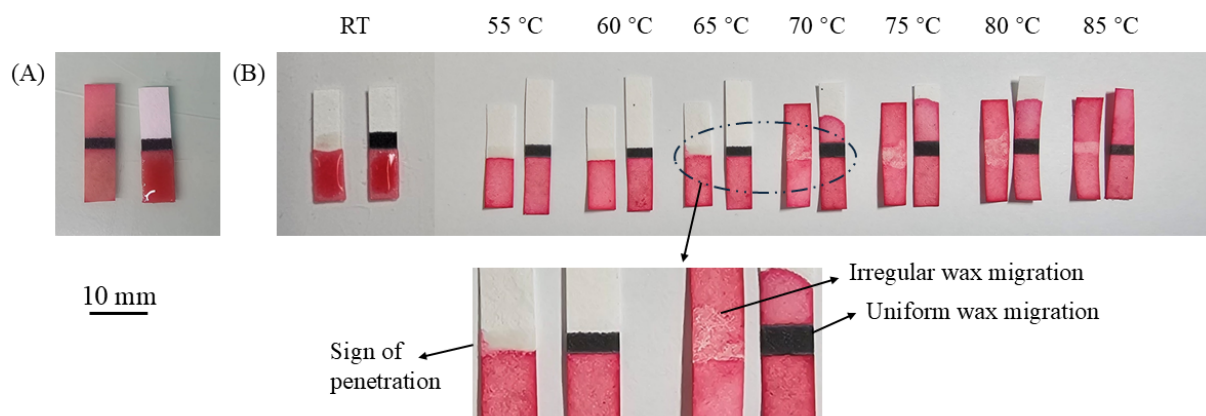


Figure 5.1: **Characterisation of wax gate fabrication and melting behaviour.** (A) Comparison of uncured (left) and cured (right) wax gates. The uncured gate failed to stop fluid flow, confirming the necessity of thermal reflow to impregnate the paper fibres. (B) Thermal threshold testing of Candelilla wax (left strip in each pair) and Paraffin-based wax (right strip) on a hot plate (1 min duration). Candelilla wax melted and opened more readily above 65 °C, while Paraffin wax retained its shape and restricts flow, expanding locally rather than migrating downstream.

wax gate (right, black strip) remained impermeable. As the temperature increased above 65 °C, the Candelilla wax exhibited superior flow properties, melting completely to allow unrestricted fluid transport to the other end of the strip. The migration of the Candelilla wax itself was visible as a moving translucent block.

In contrast, the Paraffin-based wax displayed more rigid behaviour. Rather than flowing downstream with the liquid, the Paraffin barrier tended to expand uniformly in width, restricting fluid flow even at elevated temperatures. This difference is likely attributable to the distinct rheological properties of the wax types and the deposition methods (manual application against printer ink formulation). At 85 °C, the rapid wicking of the dye water outpaced the wax migration, occupying the strip before significant wax movement could be observed.

Based on these observations, Candelilla wax was identified as more suitable for instant actuation strategies where a high-temperature spike triggers rapid opening. However, its lower melting threshold risks premature opening during the 65 °C amplification step. Paraffin-based wax, with its higher viscosity and structural stability at 65 °C, was deemed more suitable for a time-delayed actuation strategy. Its resistance to immediate flow at reaction temperatures suggests it can serve as a passive timer, holding the sample for the duration of the amplification before slowly softening to allow release.

To verify the feasibility of using the Paraffin gate as a time-delayed valve, a closed-system test was conducted to minimise evaporation and mimic device conditions. Strips containing 2 mm Paraffin gates were loaded with 20 μL of dye water, wrapped in Parafilm, and incubated at 65 °C. Figure 5.2 confirms that after 10 minutes of incubation, the Paraffin gates successfully opened, allowing fluid to cross the barrier. This result demonstrates that while the Paraffin gate restricts flow under 65 °C (as seen in the 1-minute hot plate test), it eventually opens under sustained heating, validating its potential as a programmable passive valve for the integrated device.

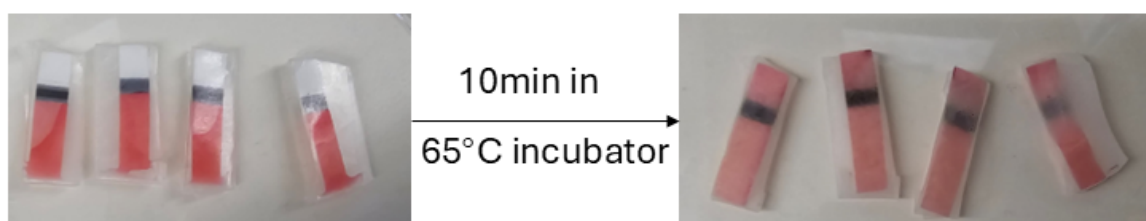


Figure 5.2: **Validation of the time-delayed actuation mechanism.** Paraffin-based wax gates (2 mm width) were loaded with dye, sealed to prevent evaporation, and incubated at 65 °C. After 10 minutes, the gates successfully opened, demonstrating that sustained heating at the LAMP reaction temperature eventually breaches the barrier, enabling passive, timer-based fluid release.

5.1.2 Geometric Tuning of Wax Gate Delay Time

To achieve a fully automated workflow, the opening of the wax valve must be synchronised with the completion of the nucleic acid amplification. Since the on-paper LAMP reaction in the device requires a 40-minute incubation, the passive valve must maintain a closed state for this duration before releasing the fluid. To calibrate this delay, the relationship between the wax gate geometry and the actuation time was investigated under realistic device conditions.

Unlike the open hot-plate tests, this experiment utilised a device-like architecture to account for the thermal mass and heat transfer characteristics of the final platform. Wax gates were fabricated on filter paper and sealed beneath a PMMA layer using PCR adhesive film, with an inlet port laser-cut for dye injection. A PTC heater was attached to the bottom of the assembly to provide a stable in-device temperature of 65°C, mimicking the thermal environment of the amplification step.

Initial testing was conducted with a 2 mm wide Candelilla wax gate to verify its suitability in a sealed environment. As shown in Figure 5.3(A), the Candelilla gate softened and allowed dye to pass through after approximately 4 minutes of heating. While this confirms that Candelilla wax can eventually open at 65°C in a closed system due to heat accumulation, the 4-minute delay is insufficient for the 40-minute LAMP reaction, leading to premature sample loss. This result reinforced the decision to utilise Paraffin-based wax, which possesses a higher viscosity in its softened state, for the time-delayed actuation strategy.

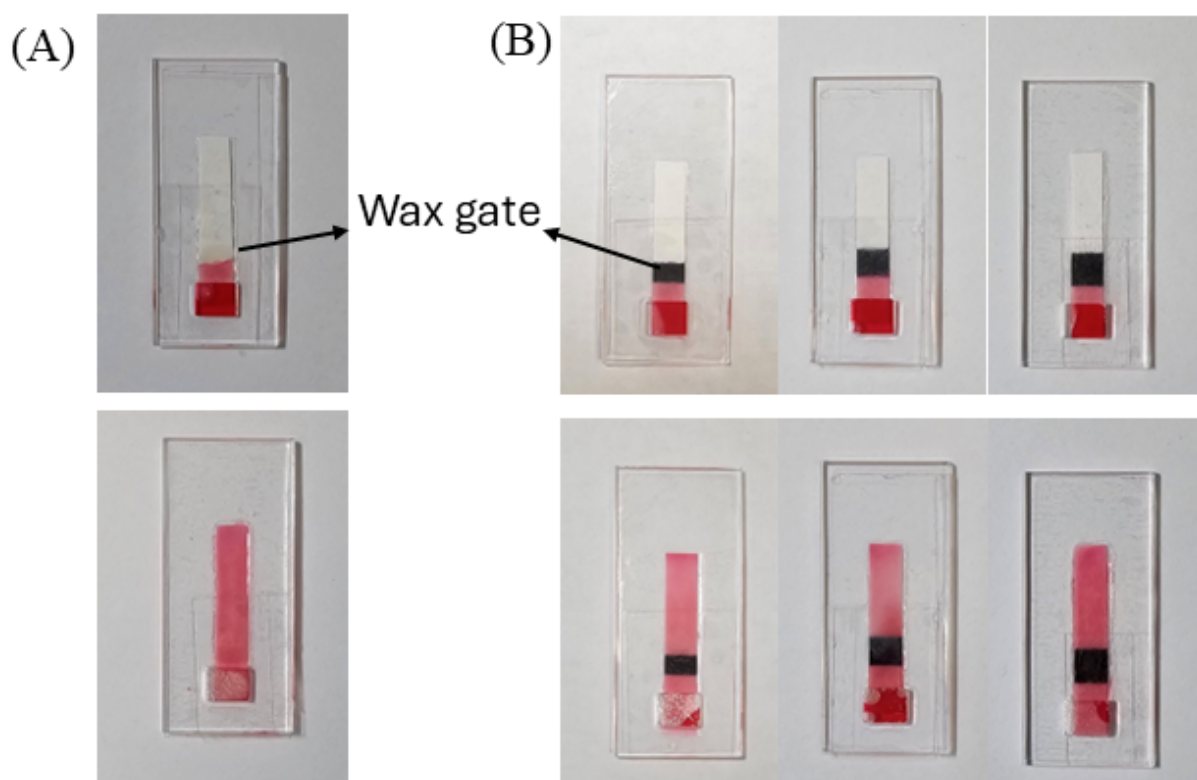


Figure 5.3: **Visualisation of wax gate actuation under sealed, in-device heating conditions at 65 °C.** (A) A 2 mm Candelilla wax gate (top: before, bottom: after) opens rapidly in 4 minutes. (B) Paraffin-based wax gates of increasing width (Left to Right: 2 mm, 3 mm, 4 mm, 5 mm). The gates remain closed initially (top row) and allow fluid passage only after specific delay times (bottom row), illustrating the tunability of the paraffin material.

Subsequently, Paraffin-based gates with widths of 2 mm, 3 mm, 4 mm, and 5 mm were evaluated to establish a tunable correlation between gate width and delay time. Figure 5.3(B) illustrates the progression of dye across the different gate widths before and after actuation. The quantitative relationship, plotted in Figure 5.4, exhibits a distinctly linear correlation between the gate width and the required actuation time. Because the paper substrate possesses a constant thickness and the fluidic channel has a fixed width, increasing the longitudinal gate width results

in a directly proportional increase in the mass (m) of the deposited wax. To validate this linear behaviour, we must consider the quantitative thermodynamic energy balance of the system. The total energy supplied by the heater must equal the energy required to raise the temperature and melt the wax, plus any thermal energy lost to the surrounding environment over the duration of the actuation.

Using an average specific heat capacity (Cp) for the material across the temperature range, the energy balance can be expressed in terms of power rates:

$$P \cdot t = m(Cp \cdot \Delta T + L_m) + P_{loss} \cdot t \quad (5.1)$$

where P is the constant electrical power of the heater, t is the heating time, ΔT is the required temperature rise, L_m is the latent heat of melting (Trigui *et al.* 2013), and P_{loss} is the rate of convective heat loss to the environment. Rearranging this equation to solve for the actuation time (t) yields:

$$t = \frac{m(Cp \cdot \Delta T + L_m)}{P - P_{loss}} \quad (5.2)$$

To determine the impact of the heat loss term (P_{loss}) on this relationship, an order-of-magnitude convective loss estimate was performed using Newton's Law of Cooling, $P_{loss} = hA\Delta T$. The heated zone possesses an exposed surface area (A) of approximately 20 mm^2 ($20 \times 10^{-6} \text{ m}^2$). Assuming a conservative natural convection coefficient for static air of $h \approx 15 \text{ W}/(\text{m}^2 \cdot \text{K})$ and a maximum temperature differential (ΔT) of 40 K, the rate of heat loss per exposed face is (Incropera *et al.* 2011):

$$P_{loss,face} = 15 \cdot (20 \times 10^{-6}) \cdot 40 \approx 0.012 \text{ W} \quad (5.3)$$

Accounting for bilateral environmental exposure (both the top and bottom of the paper), the total maximum convective loss is $P_{loss,total} \approx 0.024 \text{ W}$. When compared to the 0.5 W input power (P) of the printed heater, this environmental loss represents less than 5% of the total supplied energy.

Because P_{loss} is exceptionally small and stable, the denominator of the time equation ($P - P_{loss}$) functions as a static constant. Similarly, the intrinsic material properties in the numerator (Cp , ΔT , and L_m) remain unchanged. Consequently, the equation simplifies to show that the heating time (t) is strictly and directly proportional to the mass (m) and therefore the gate width.

This near-adiabatic theoretical scaling is reflected in the experimental data. The 2 mm gates provided a mean actuation time of 7.6 ± 0.4 minutes ($n = 3$), offering a short delay suitable for brief incubation steps but inadequate for LAMP. Increasing the width to 3 mm extended the delay to 25.6 ± 1.5 minutes. The 4 mm gates achieved a mean actuation time of 42.1 ± 2.6 minutes. This duration aligns perfectly with the standard amplification protocol, providing a 40-minute reaction window followed by automatic downstream transport without any user intervention. Finally, the 5 mm gates demonstrated an extended delay of 58.7 ± 3.2 minutes. While this confirms the linear scalability of the timing mechanism, the near-hour wait time was deemed unnecessary for the current assay. Consequently, the 4 mm Paraffin-based wax gate was selected as the flow control element for the integrated device.

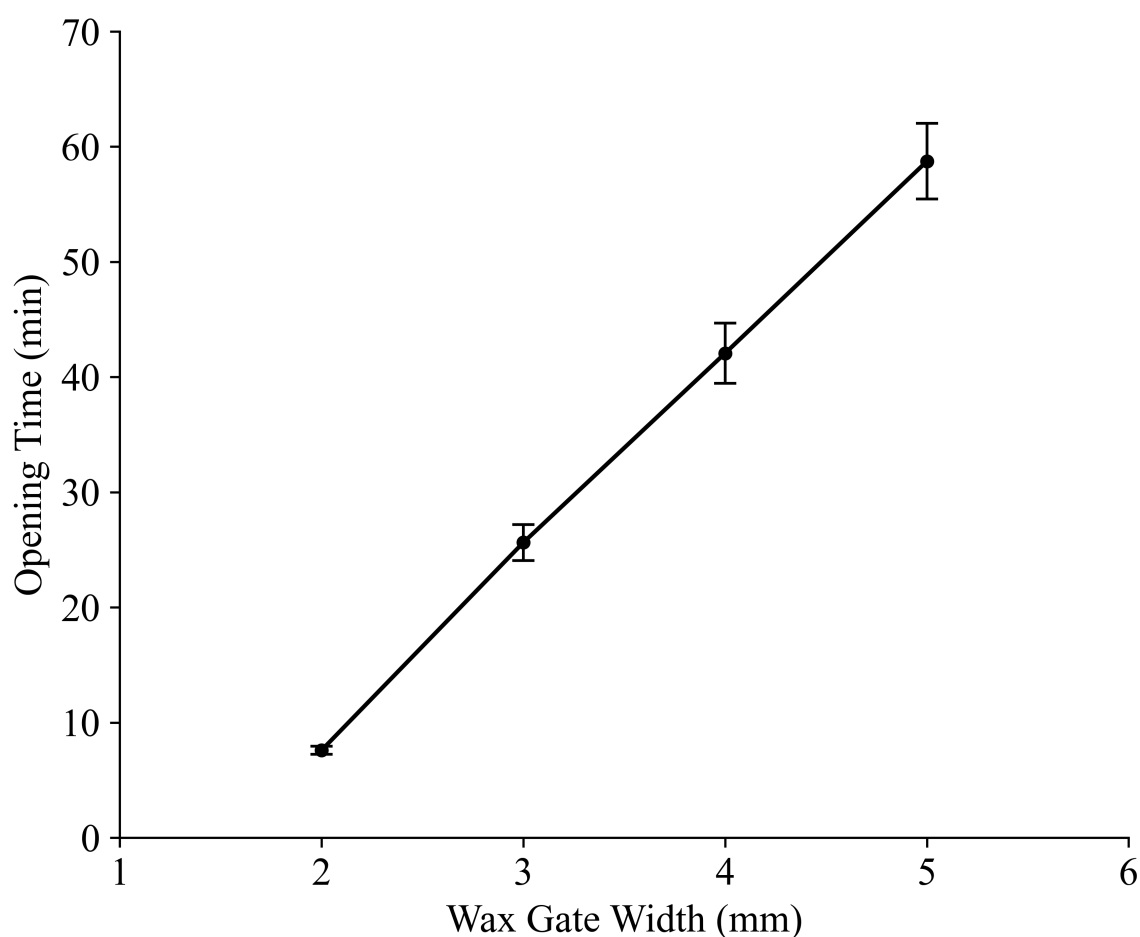


Figure 5.4: **Quantitative relationship between the width of the Paraffin-based wax gate and the passive actuation time at 65 °C.** Data points represent the mean opening time ($n = 3$), with error bars indicating standard deviation. A clear trend is observed, with the 4 mm gate providing a delay of approximately 42 minutes, matching the required window for on-chip LAMP amplification.

5.1.3 Initial Integrated Device Demonstration

Following the characterisation of the individual components, an initial integration study was conducted to validate the compatibility of the PTC heating element with the microfluidic architecture and to assess the fluidic requirements of the system. Two prototype configurations, designated Device A and Device B (detailed in Section 2.7.1), were employed for these preliminary tests.

Device A, designed to verify the electrothermal and biochemical interface, was first evaluated to confirm that the PTC-based heater could sustain a successful LAMP reaction within the PMMA chamber. The reaction chamber was loaded with 25 μL of LAMP reagents containing 10^5 copies of Influenza B template, while the buffer chamber was loaded with 40 μL of running buffer. Following the 40-minute amplification powered by the PTC heater, the buffer was manually released to transport the amplicons to the lateral flow strip. The results, shown in Figure 5.5(A), demonstrated clear positive signals for all three replicates and a clean result for the negative control. This confirms that the thermal profile generated by the PTC heater is biochemically valid for LAMP amplification within the specific geometry of the microfluidic chip and that the detection module functions correctly downstream.

Subsequently, Device B was utilised to evaluate the feasibility of instant actuation using a Candelilla wax gate and to determine if the device could operate without a separate buffer addition step. In this configuration, the Candelilla wax gate was subjected to the direct heat of the PTC element. The gate successfully opened within approximately 2 minutes of heating. This rapid actuation is attributed to the thermal gradient of the system; as established in Chapter 3, the surface temperature of the PTC heater is slightly higher than the equilibrium liquid temperature of 65 °C. Since Candelilla wax melts and flows readily just above 65 °C (as observed in Section 5.1.1), the localised surface heat triggered the valve shortly after activation.

However, a critical fluidic limitation was observed during the operation of Device B. As illustrated in Figure 5.5(B, left), although the wax gate opened and allowed the reaction mixture to pass, the fluid front stopped on the sample pad of the lateral flow strip. The 25 μL volume of the LAMP reaction was insufficient to saturate the combined void volume of the fluidic pathway

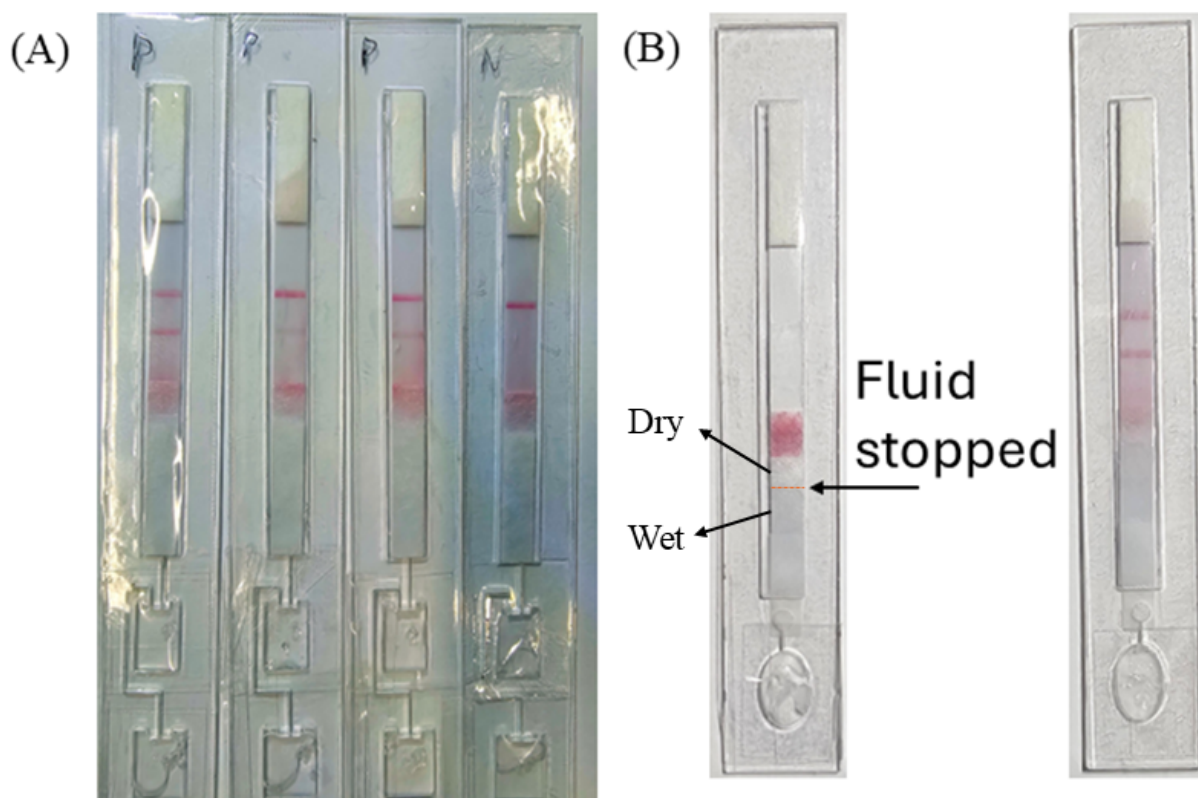


Figure 5.5: **Initial validation of integrated device prototypes.** For scale, all lateral flow strips depicted measure 4 mm x 60 mm. (A) Results from Device A, confirming that the PTC heater successfully drives LAMP amplification (3 positives, 1 negative) and that the product is detectable on the strip. (B) Results from Device B using a Candelilla wax gate. The left image shows the gate opening, but the fluid front stalls at the sample pad (arrow) due to insufficient volume (25 μL). The right image shows successful signal development only after the manual addition of a running buffer, proving the necessity of a buffer delivery mechanism.

and drive capillary transport across the detection membrane. To theoretically validate this fluidic stalling, the minimum bed volume required to wet the materials was calculated based on the geometric dimensions of the 4 mm wide strip and the standard porosity capacities of the selected substrates (Merck Millipore 2019, Koczula *et al.* 2016).

The fluidic pathway consists of the cellulose filter paper ($4\text{ mm} \times 10\text{ mm} = 0.40\text{ cm}^2$), the glass fibre sample pad ($4\text{ mm} \times 17\text{ mm} = 0.68\text{ cm}^2$), the conjugate pad ($4\text{ mm} \times 8\text{ mm} = 0.32\text{ cm}^2$), and the nitrocellulose detection membrane ($4\text{ mm} \times 25\text{ mm} = 1.00\text{ cm}^2$). Based on standard manufacturer fluid retention specifications, cellulose filter paper retains approximately $15\text{ }\mu\text{L}/\text{cm}^2$, highly porous glass fibre pads retain roughly $35\text{ }\mu\text{L}/\text{cm}^2$, and capillary nitrocellulose membranes hold about $10\text{ }\mu\text{L}/\text{cm}^2$. Applying these capacities yields the following minimum volume requirements: $6.0\text{ }\mu\text{L}$ to saturate the filter paper, $23.8\text{ }\mu\text{L}$ for the sample pad, $11.2\text{ }\mu\text{L}$ for the conjugate pad, and $10.0\text{ }\mu\text{L}$ to clear the nitrocellulose membrane.

Summing these individual void volumes reveals that a theoretical absolute minimum of 51.0 μL of fluid is required simply to saturate the materials up to the end of the detection zone. Therefore, the 25 μL amplicon input, which is almost just saturating the initial filter paper (6.0 μL) and the sample pad (23.8 μL), is mathematically incapable of reaching the conjugate pad. This completely aligns with the physical stalling observed in the prototype.

A valid test result was only achieved after the manual addition of running buffer, which provided the necessary excess volume to elute the trapped amplicons and flush them across the detection zone (Figure 5.5B, right). Subsequent fluidic testing determined that an auxiliary running buffer volume of at least 50 μL is required in this configuration to ensure the liquid front successfully traverses the wax gate, fully saturates the lateral flow strip, and reaches the absorbent wicking pad. This fundamental bed volume deficit definitively proves that the limited volume of the amplification reaction cannot drive the lateral flow test alone, highlighting the absolute necessity of integrating a secondary buffer-delivery mechanism into the final device architecture.

5.1.4 Investigation of Fluidic Bypassing and Edge Effects

To implement the time-delayed actuation strategy validated in Section 5.1.2, the Paraffin-based wax gate paper strip was integrated into the PMMA device architecture. The dimensions and layout of this initial prototype are detailed in Figure 5.6(A). As illustrated in the cross-sectional side view, this design features a sandwiched configuration where the paper strip is compressed between the rigid PMMA base and the adhesive PCR sealing film. This geometry creates distinct physical interfaces along the longitudinal edges of the porous strip where the paper, plastic wall, and adhesive film meet.

However, testing with LAMP reagents revealed a critical failure mode: fluidic bypassing. Instead of being contained by the internal hydrophobic wax barrier, the liquid migrated along the external edges of the paper strip at the interface with the sealing film, effectively short-circuiting the valve. Figure 5.6(B) illustrates the two situations of this issue. In some instances (Figure 5.6B, left), partial bypassing occurred where a portion of the liquid leaked past the wax barrier along the edges but eventually stalled at the distal end of the paper strip or the very front of the sample pad. This bypassing resulted in considerable sample loss, leaving insufficient reagent volume

to be amplified, leading to faint or no signals for positive samples after 40 mins amplification. In more severe cases (Figure 5.6B, right), immediate bypassing occurred upon pipette loading, where the reagent rapidly wicked along the edges, short-circuiting the wax gate entirely and prematurely wetting the membrane before amplification could take place.

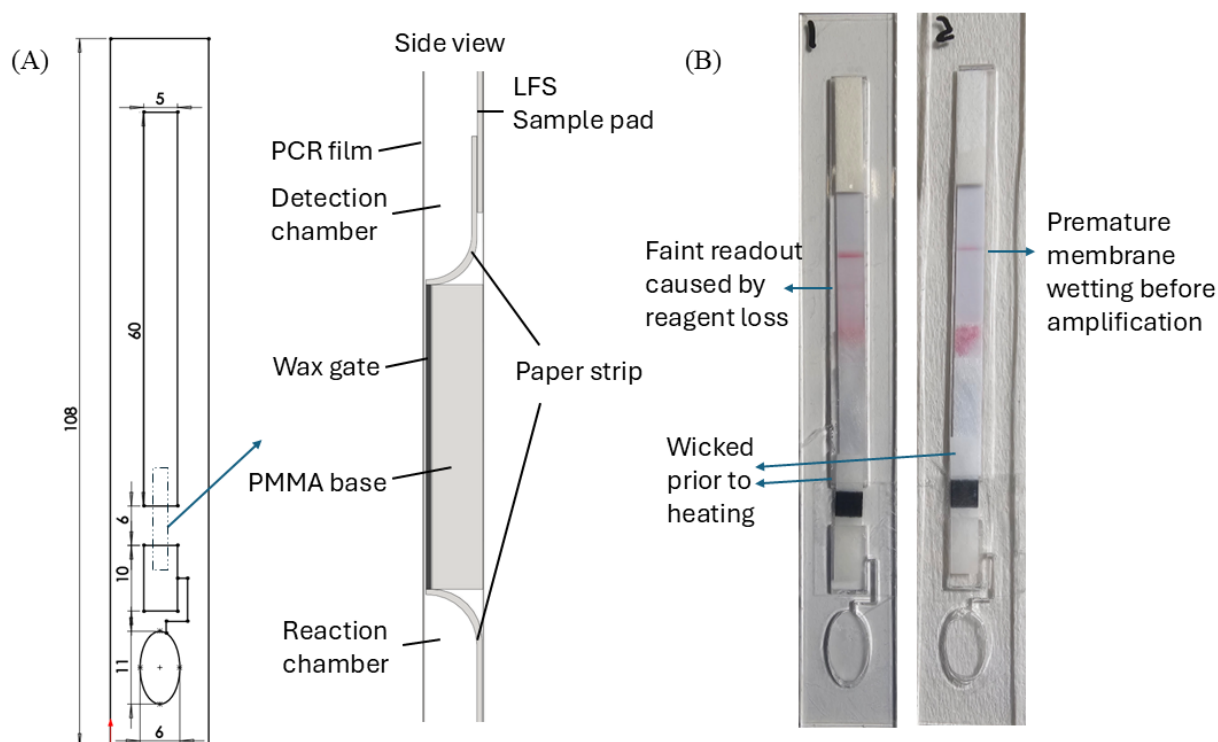


Figure 5.6: **Design and failure analysis of the initial integrated device.** (A) Schematic of the prototype dimensions and assembly. The cross-sectional side view highlights the sandwiched configuration, where the paper strip is compressed between the PMMA base and the adhesive sealing film, creating capillary interfaces at the edges. (B) Observation of fluidic bypassing in devices loaded with LAMP reagents. The left device shows partial bypassing where liquid leaks past the gate but stops at the sample pad interface, causing sample loss and a faint result after amplification. The right device shows severe, immediate bypassing upon loading, where reagents rapidly wick along the paper edge, short-circuiting the wax gate entirely.

To isolate the cause of this failure, the influence of fluid properties and loading pressure was investigated (Figure 5.7). Initial tests showed that the dye solution was successfully contained by the wax gate without edge leakage (Figure 5.7A, right). However, when the sample was switched to deionised (DI) water, leakage along the edge occurred consistently (Figure 5.7A, left). It was initially hypothesised that pneumatic pressure generated during pipette loading might be compromising the adhesion between the film and the substrate, creating micro-channels along the

paper edge. To test this, pressure relief vents were introduced into the various chambers using a biopsy punch. As shown in Figure 5.7B, these vents successfully prevented bypassing when using DI water, suggesting that pressure equalisation could resolve the issue for simple aqueous solutions.

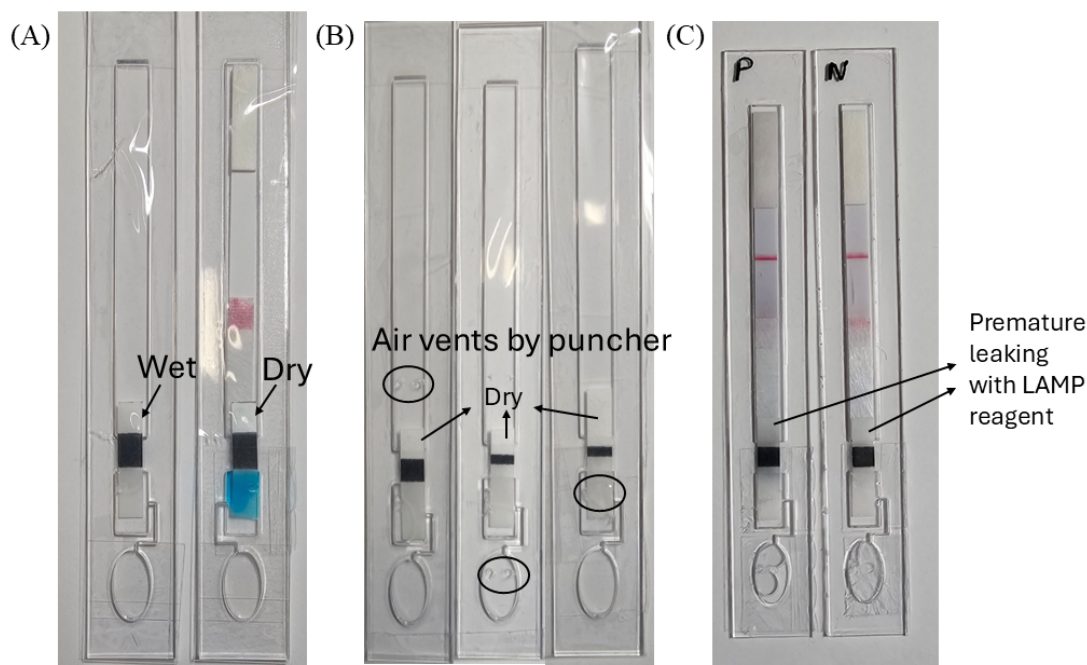


Figure 5.7: **Investigation into the causes of interfacial bypassing.** (A) Comparison of fluid types in sealed devices: Deionised water leaks along the edge (left), while dyed water is successfully contained (right), indicating surface tension differences. (B) Effect of pressure relief: Punching air vents in different chambers eliminates bypassing when using DI water. (C) Failure with LAMP reagents: Despite the use of air vents, LAMP reagents still bypass the gate due to their high wettability, resulting in assay failure (no signal).

However, when these pressure-vented devices were tested with the actual LAMP reaction mixture, bypassing persisted, and no valid signals were observed (Figure 5.7C). This critical discrepancy (vents fixed the issue for water but not for LAMP reagents) indicates that the physicochemical properties of the fluid are the dominant failure mechanism. The LAMP master mix exhibits higher wettability (lower surface tension) than pure water or simple dye solutions. This property allows the reagent to wick into microscopic capillaries at the paper/film/PMMA interface (visible in the side view of Figure 5.6A) that water would otherwise not penetrate, regardless of the internal chamber pressure. Consequently, the edge-sealing approach was proved unreliable for containing the LAMP reagents, necessitating a fundamental redesign of the fluidic interface.

5.1.5 Optimisation of Chamber Geometry and Reaction Volume

To eliminate the edge-wicking path responsible for the fluidic failure described in the previous section, the device geometry was adapted. The physical PMMA barrier separating the reaction zone from the detection zone was removed, creating a single continuous chamber bridged by the paper strip. Figure 5.8(A) details the dimensions of this connected chamber configuration. In this design, the wax gate is not compressed against a sealing edge at the valve location; instead, it resides freely within the channel, removing the capillary interface responsible for bypassing.

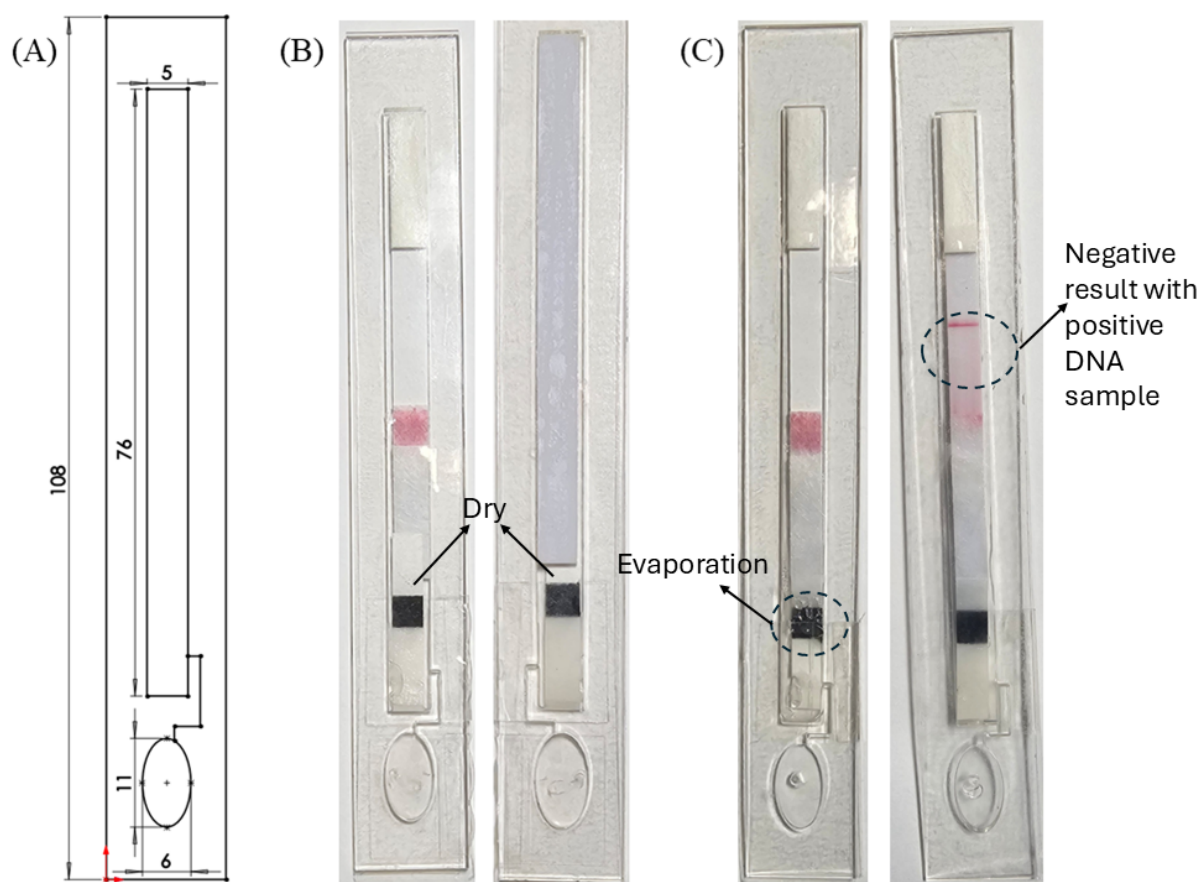


Figure 5.8: **Design and testing of the connected chamber geometry.** (A) Dimensions of the initial connected device where the barrier between zones is removed. (B) Bypassing test showing successful containment of reagents (no leakage) from both front and back views. (C) Functional test failure: Evaporation during the 40-minute incubation leads to insufficient fluid transport and thus the amplification failed, causing no signal generation.

Initial validation of this new geometry with LAMP reagents confirmed the elimination of the leakage issue. As shown in Figure 5.8(B), no fluid migration was observed from either the front or the back of the device before thermal actuation, validating the structural integrity of the seal. However, functional testing with the standard 25 μ L reaction volume revealed a new chal-

lenge. The removal of the barrier increased the headspace volume within the chamber, leading to more severe evaporation during the 40-minute incubation at 65 °C. Figure 5.8(C) illustrates the significant condensation observed on the film and the subsequent failure of the assay. Despite the successful thermal hold, no test line signal was generated. This failure is due to the large surface-to-volume ratio of the open chamber, which facilitated rapid solvent loss during the 40-minute incubation, leading to reaction dry-out and failure (C. Zhang *et al.* 2009).

To compensate for these evaporative losses and ensure robust fluid transport, the reaction-detection chamber was elongated by 5 mm, and the buffer chamber dimensions were scaled up by increasing the length from 11 mm to 13 mm and the width from 6 mm to 8 mm (specific dimensions are detailed in Chapter 2, Section 2.7.4). This expansion allowed the chamber to accommodate larger fluid volumes without the risk of overflowing.

An intermediate reaction volume of 37.5 μL (1.5 standard reactions volume, component concentrations remain unchanged) was first evaluated in this elongated design. While the fluidic transport was successful, the analytical LoD was compromised. As shown in Figure 5.9(A), while a strong signal was obtained at 1.5×10^4 copies/reaction, the signal at 1.5×10^3 copies/reaction was extremely faint or inconsistent. This represents a significant drop in LoD compared to 100 copies established in Chapter 4. This gap suggests that while 37.5 μL provided sufficient volume for wicking, it did not provide a large enough buffer against evaporation to maintain optimal biochemical conditions for low-copy amplification.

Consequently, the reaction volume was doubled to 50 μL (2 standard reactions volume, comprising 10 μL template, 10 μL primer mix, and 30 μL master mix).

The performance of the system with the 50 μL reaction volume is presented in Figure 5.9(B). A clear, distinct test line was observed at 2×10^3 copies/reaction, indicating that the increased volume successfully mitigated the concentration effects of evaporation and restored assay performance. Crucially, a control device loaded with this larger volume but kept at room temperature showed no passive leakage (Figure 5.9B, right). This confirms that the wax gate is mechanically robust enough to hold the increased hydrostatic pressure of the 50 μL volume for the duration of the assay, releasing it only upon thermal actuation.

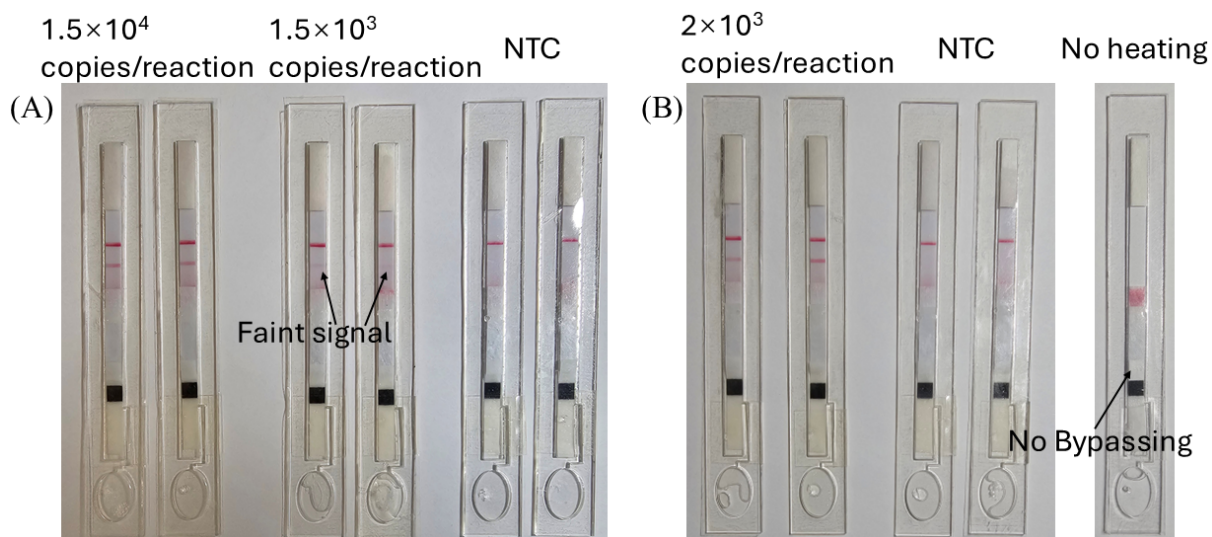


Figure 5.9: **Optimisation of reaction volume in the elongated chamber.** (A) Testing with 1.5 reactions ($37.5 \mu\text{L}$): While high concentrations (1.5×10^4 copies) are detected, the signal is poor at 1.5×10^3 copies (faint signal), indicating residual evaporation effects. (B) Testing with 2 reactions ($50 \mu\text{L}$): Performance is restored with a clear signal at 2×10^3 copies. The unheated control (right) demonstrates no passive bypassing, confirming valve integrity under the increased fluid load.

5.1.6 Analytical Performance of the Optimised Integrated Device

Following the optimisation of the chamber geometry and reaction volume, the analytical LoD of the integrated device was evaluated to determine its LoD. Using the optimised elongated device configuration ($50 \mu\text{L}$ reaction volume), the device was tested with the same serial dilutions of Influenza B synthetic DNA used in the benchtop study in Section 4.1.2. For this integrated assay, a $10 \mu\text{L}$ sample template was utilised, resulting in an absolute input load ranging from 2×10^5 down to 2 copies per reaction.

The lateral flow readouts from this LoD panel are presented in Figure 5.10. The device demonstrated robust performance across a broad dynamic range. Clear and distinct test lines were consistently observed for template concentrations from 2×10^5 down to 2×10^2 copies per reaction. The signal intensity at 2×10^2 copies remained easily distinguishable by the naked eye, indicating high amplification efficiency within the paper-based reaction chamber.

At the limiting dilution of 20 copies per reaction, stochastic amplification behavior was observed, with a clear test line appearing in one out of three replicates. No test line signal was detected at 2 copies per reaction or in the NTC, validating the system's specificity and the absence of contamination within the sealed fluidic pathway.

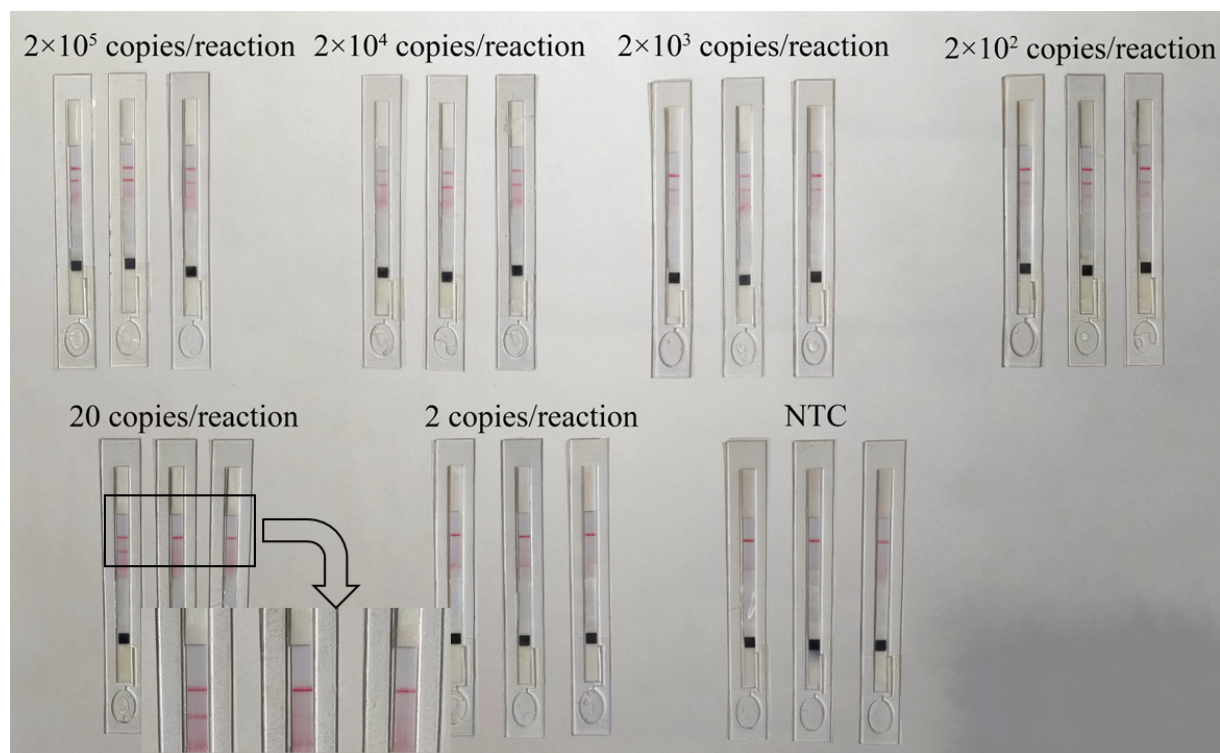


Figure 5.10: **LoD analysis of the integrated device for Influenza B detection.** The assay demonstrated consistent positive results from 2×10^5 down to 2×10^2 copies/reaction. At the limiting dilution of 20 copies/reaction, stochastic amplification is observed (1/3 positive), while 2 copies/reaction and the NTC show no non-specific signal. This performance matches the LoD of the benchtop assay (Section 4.1.2), confirming successful on-chip integration.

Crucially, the LoD magnitude achieved on the integrated platform aligns closely with the benchtop assay results established in Section 4.1.2, where the reliable LoD was determined to be 100 copies. The detection of 2×10^2 copies in the double-volume integrated device is equivalent to detecting 100 copies in a standard single-volume reaction. This confirms that the integration of the LAMP reaction into the paper-based chamber does not compromise the assay's intrinsic performance. The device successfully retains the superior LoD of the underlying chemistry while offering a streamlined, user-friendly format.

5.2 Discussion

5.2.1 Passive Synchronisation of Fluidic Control

The integration of automated fluid handling into portable diagnostic devices typically necessitates a trade-off between complexity and performance. While active microfluidic systems utilising external syringe pumps or electromechanical valves offer precise control, they require bulky external equipment that undermines the advantages of point-of-care testing (Khanjani *et al.* 2025). The device developed in this work addresses this challenge by implementing a thermal coupling strategy, where the heat source required for the biochemical reaction simultaneously serves as the actuation mechanism for the fluidic valve.

The results from Section 5.1.2 demonstrate that the actuation time of the Paraffin-based wax gate is not random but follows a linear dependence on the gate width (Figure 5.4). This geometric tunability allowed for the precise calibration of the wax gate to open after approximately 42 minutes (using a 4 mm width), strictly synchronising the fluid release with the completion of the 40-minute LAMP amplification. This passive synchronisation eliminates the need for on-chip sensors or microcontrollers to trigger the flow while the timing is intrinsically encoded in the device geometry.

Furthermore, this Paraffin-based approach offers distinct advantages over other passive valve mechanisms reported in the literature, such as dissolvable sugar matrices (Houghtaling *et al.* 2013) or capillary valves (Ghosh *et al.* 2020). Dissolvable valves are often sensitive to ambient humidity and storage conditions, leading to premature degradation, while capillary valves are sensitive to both manufacturing precision and sample properties, and typically require more sophisticated fabrication techniques (Gong *et al.* 2017, S. Wang *et al.* 2021). In contrast, the wax gate remains chemically inert and structurally solid at room temperature, ensuring robust containment during storage and sample loading (Díaz-González *et al.* 2016). The valve transitions to the open state only when the specified thermal threshold is reached, preventing premature downstream migration of unamplified reagents and thereby reducing false-negative results in lateral flow detection.

5.2.2 The Mechanics of Interfacial Bypassing

The transition from the initial “sandwiched” device to the final “connected” chamber architecture was driven by a critical failure mode identified as interfacial bypassing. In the initial design, fluidic containment relied on the wax barrier being compressed between the PMMA substrate and the adhesive sealing film. While this geometry theoretically seals the channel, experimental results in Section 5.1.4 revealed that this interface acts as a preferential flow path, particularly for the LAMP reaction mixture.

This failure can be attributed to the edge effect. When a porous strip is embedded within a rigid channel, microscopic gaps inevitably form along the longitudinal edges where the paper, the plastic wall, and the sealing film meet (Songok *et al.* 2016). The ability of a fluid to penetrate these gaps is governed by its wettability and contact angle with the substrate materials (Carstens *et al.* 2016). While deionised water exhibits a relatively high contact angle and surface tension, allowing it to be contained by the hydrophobic wax barrier (especially when pneumatic pressure is relieved), the LAMP reaction mixture behaves differently.

Although the LAMP reagents do not contain added surfactants, the isothermal master mix is a complex formulation containing a GspSSD2.0 DNA polymerase, thermostable inorganic pyrophosphatase, $MgSO_4$, dNTPs, and a ds-DNA binding dye (OptiGene Ltd. 2019). This composition, rich in proteins and salts, alters the physicochemical interactions at the solid-liquid interface, leading to increased wettability compared to pure water (Y. Jia *et al.* 2005, K. Wang *et al.* 2024). This enhanced wetting capability allows the reagent to spontaneously wick into the microscopic capillaries formed at the strip edges, thereby bypassing the hydrophobic wax barrier through the path of least resistance.

The connected chamber design (Section 5.1.5) resolved this by fundamentally altering the boundary conditions of the valve. By removing the physical PMMA barrier above the wax gate, the device eliminated the tight geometry responsible for the edge effect. In this configuration, the wax gate resides freely within the fluidic channel. Without a narrow capillary interface to wick along, the fluid front is forced to move through the hydrophobic wax gate. In this state, the paraffin wax gate successfully held the fluid flow until the thermal actuation threshold was reached. This finding underscores the necessity of designing fluidic geometries that account for the specific physicochemical properties of biological reagents, rather than just relying on hydraulic principles derived from water-based models.

5.2.3 Thermodynamic Stability and Analytical LoD

The second major engineering challenge addressed in this chapter was the thermodynamic instability of the amplification reaction within the connected chamber design. While removing the physical barrier solved the fluidic bypassing issue, it inadvertently created a geometry with a high surface-to-volume ratio and increased headspace. As observed in Section 5.1.5, this configuration was highly susceptible to evaporative losses during the 65°C incubation.

In microfluidic systems, evaporation is not merely a loss of fluid; it also serves as a dynamic mechanism of chemical inhibition. As water evaporates from the reaction mix, the concentration of solutes (specifically Magnesium ions (Mg^{2+}) and other buffer salts) spikes proportionally. It is well established that deviating from the optimal ionic strength introduces severe drawbacks to isothermal amplification. Higher divalent salt concentrations, in particular, can inhibit LAMP primer annealing and enzyme function (Nwe *et al.* 2024, Purohit *et al.* 2024). Excess magnesium can decrease the fidelity and specificity of the DNA polymerase, as well as interfere with the strand displacement mechanism by excessively stabilising the double-stranded DNA template. Furthermore, elevated Mg^{2+} levels can lead to primers annealing to incorrect sites (non-specific binding) rather than the target sequence, blocking the specific amplification pathway required for signal generation (Kuffel *et al.* 2020, J. Liu *et al.* 2013).

The optimisation trials involving reaction volume scaling provided empirical evidence of this concentration-dependent failure mode. Initial tests with the standard 25 μL volume resulted in total assay failure. Increasing the volume to 37.5 μL (1.5 standard reactions) partially restored function but failed with lower concentrations (1.5×10^3 copies), suggesting that while it provided sufficient fluid for wicking, its performance was compromised due to evaporation.

By further increasing the volume to 50 μL (2 standard reactions), the system achieved a critical mass that buffered against these concentration shifts. Even with unavoidable micro-evaporation, the bulk volume remained sufficient to maintain salt and Mg^{2+} levels within the active window for the enzyme. Consequently, the device achieved a Limit of Detection (LoD) of 200 copies (total load). When normalised to the input volume, this LoD is equivalent to detecting 100 copies in a standard single-volume reaction, matching the benchmark LoD established in Chapter 4.

Crucially, this result determined the endpoint of the volume optimisation process. Further expansion of the reaction volume (e.g., to 2.5 or 3 standard reactions) was not pursued. Since the 50 μL configuration successfully replicated the maximum analytical LoD of the optimised benchtop protocol, increasing the volume further would not produce significant performance improvement. Instead, it would only increase reagent consumption and fluid handling complexity, undermining the cost-effectiveness and portability goals of the point-of-care device. The 50 μL volume therefore represents the optimal engineering balance: large enough to buffer against thermodynamic instability, yet small enough to remain efficient.

5.3 Contribution to Knowledge

In this chapter, we have presented the development of a fully passive, paper-based microfluidic platform that synchronises biochemical amplification with fluidic transport through a novel thermal coupling strategy. By utilising the 65°C heat of the LAMP reaction to actuate a phase-change paraffin gate, we established that complex fluidic sequences can be executed without extra external power or electronic controllers. Our work provides a foundational design rule for paper microfluidics by demonstrating a precise linear relationship between valve width and actuation timing (Around 13.8 *min/mm*). This finding allows for the geometric programming of diagnostic protocols, where the specific delay required for amplification is intrinsically encoded into the device architecture through simple layout modifications.

Beyond mechanical actuation, we provided a detailed characterisation of interfacial bypassing, identifying it as a critical failure mode in hybrid paper-polymer devices. We demonstrated that the complex, protein-rich matrix of the LAMP reagents exhibits enhanced wetting properties that allow reagents to wick along microscopic capillary interfaces between the paper and the device housing. By identifying that this edge effect is driven by interactions at the rigid-porous boundary, we developed a connected chamber architecture which removes the compressive sealing interface at the wax gate, forcing the fluid front to interact directly with the hydrophobic wax area and providing a robust solution for reagent containment.

Furthermore, we established essential thermodynamic scaling rules for translating isothermal assays from closed tubes to open-chamber paper formats. We found that the high surface-to-volume ratio in paper-based reaction zones necessitates a higher reaction volume to ensure amplification. Our research showed that doubling the reaction volume to 50 μL successfully prevents heat-induced evaporation from concentrating buffer salts and divalent cations to inhibitory levels. This strategy ensures that Mg^{2+} concentrations remain within the optimal window, preventing the loss of polymerase fidelity and the non-specific primer annealing.

Finally, we confirmed that this integrated approach preserves the analytical detection limit of the assay, achieving an LoD of 200 copies/reaction (equivalent to 20 copies/ μL). By matching the performance of the benchmark protocol established in Chapter 4, we proved that paper-matrix chambers are fully biocompatible and capable of supporting high-efficiency amplification. We also justified the optimisation endpoint, establishing that once parity with the benchtop assay is reached, further volume increases provide no additional performance gain. This comprehensive integration of fluidic timing, interfacial engineering, and biochemical scaling provides a clear framework for the development of autonomous and integrated diagnostic devices.

Conclusions and Future Works

6.1 Conclusions

This thesis developed and evaluated a paper-based LAMP–lateral-flow platform for nucleic-acid detection using synthetic respiratory-virus DNA templates. The work established the thermal, fluidic, material, and lateral-flow design principles required to combine isothermal amplification with visual lateral-flow readout in a low-cost integrated device format. The main conclusions are as follows:

1. A paper-compatible PTC-ink heater can provide low-voltage thermal management for LAMP when the high sheet resistance of the PTC material is compensated by an appropriate silver interdigitated-electrode design. The interdigitated geometry reduced the effective current path through the PTC layer and enabled the resistance of the heater to be tuned into a range suitable for portable power sources. The optimised heater achieved LAMP-compatible liquid temperatures and showed passive self-regulating behaviour arising from the positive temperature coefficient response of the carbon-based composite ink. This demonstrates that printed PTC materials can be used as a low-complexity alternative to actively controlled resistive heaters for isothermal amplification.
2. Heater geometry was the dominant factor controlling the electrical and thermal performance of the printed PTC device. Electrode gap spacing, active heating area, and the continuity of the PTC coating all affected the initial resistance, heating rate, equilibrium temperature, and thermal uniformity. The results show that a continuous PTC layer on cellulose paper, combined with silver interdigitated electrodes, provided the most practical architecture for the device developed in this thesis. The observed batch-to-batch variation also indicates that fabrication consistency is a key design consideration for future production of printed diagnostic heaters.

3. The dot-array lateral-flow format improved the practicality of multiplex nucleic-acid read-out compared with the conventional multi-line format. In the line-format strip, sequential capture regions were more susceptible to spatial crowding and downstream signal depletion, particularly when high-yield LAMP products were present. The dot-array layout provided a more compact capture architecture and allowed multiple respiratory-virus amplicons to be visually distinguished on a single strip. This establishes dot-array patterning as a useful strategy for increasing the information density of nucleic-acid lateral-flow strips without simply extending the strip length.
4. Cellulose filter paper was more suitable than glass fibre for the on-paper LAMP and amplicon-release workflow used in this thesis. The reaction matrix in an integrated device must support amplification while also allowing amplified products to be transferred into the lateral-flow strip. The results indicate that substrate choice directly affects both amplification compatibility and downstream detection. In this device configuration, cellulose filter paper provided a more effective balance between reaction support and product release, whereas glass fibre retained DNA products more strongly and was less suitable for direct transfer into the lateral-flow readout.
5. The thermally actuated wax gate provided an effective passive timing mechanism for coupling LAMP amplification with lateral-flow detection. The wax barrier maintained separation between the reaction zone and the detection strip during incubation and opened under the thermal conditions generated for amplification. The relationship between wax-gate width and actuation delay showed that timing could be programmed geometrically, allowing fluid release to be synchronised with the amplification step without pumps, sensors, microcontrollers, or manual valve operation.
6. The integrated device demonstrated that passive heating, on-paper amplification, wax-gate timing, and lateral-flow detection can be combined into a single analytical workflow. The final device detected Influenza B synthetic DNA with an observed limit of detection of 200 copies total input under the tested conditions. This result indicates that integration into the paper-based device did not substantially compromise the analytical detection performance of the underlying LAMP–lateral-flow chemistry. The device-level experiments also showed that successful integration depends on interfacial sealing, chamber geometry, reaction volume, evaporation control, and reliable contact between the amplification zone and the detection strip.

These findings establish three main contributions: a printed self-regulating PTC-ink heater for passive LAMP thermal management, a dot-array nucleic-acid lateral-flow format for compact multiplex readout, and a thermally actuated wax gate for passive fluidic timing. The work therefore provides an integrated device framework for future paper-based molecular diagnostics. For practical implementation, the most important additional requirement is the integration of upstream sample preparation so that nucleic acids from real respiratory specimens can be introduced into the amplification workflow. This implementation step is addressed further in the Future Work section.

6.2 Future Works

The development of the integrated paper-based diagnostic platform presented in this thesis marks a significant step toward instrument-free molecular testing. However, to translate this prototype into a commercially viable and clinically robust product, several engineering and biochemical challenges must be addressed. The future development of this technology is envisioned in two distinct phases: short-term objectives, achievable within a shorter time period, to refine and validate the current architecture; and long-term strategic goals, aimed at revolutionising the device's power source, manufacturability, and user interface.

6.2.1 Short-Term Objectives: Integration and Validation

Implementing Multiplexing in the Integrated Device

While Chapter 4 successfully validated the performance of the micro-dot array for the simultaneous detection of five respiratory pathogens (Influenza A/B, RSV A/B, and SARS-CoV-2), and Chapter 5 demonstrated the fluidic automation of the integrated device using a single-target model, the immediate next step is to merge these two achievements. The current integrated prototype utilises a standard line-format strip. The short-term goal is to transfer the optimised 5-target dot array into the final chamber design.

To achieve this, we propose a parallel-chamber architecture. Utilising the multiplex primer sets established in Chapter 2 (Table 2.4), which allow for the co-amplification of Influenza A/B and RSV A/B in single reactions, the device requires only three distinct reaction chambers (Influenza, RSV, and COVID) rather than five. Given that a single amplification zone (including

the wax gate) measures 4×18 mm, three such chambers can be stacked in parallel with minimal spacing to fit entirely within the footprint of the existing 19×18 mm PTC heater. This configuration allows for simultaneous heating without modifying the power source. To visualise the results on a single multiplexed lateral flow strip, a fork-like paper pathway will be designed to merge the fluidic streams from the three independent chambers into one detection zone. Future experiments will focus on re-evaluating the flow dynamics of this convergent path, specifically adjusting the reaction and buffer volumes to ensure that the merged flow uniformly saturates the high-density dot array without introducing backflow or artifacts that could compromise detection.

Validation with Clinical Samples

To date, the analytical detection performance of the platform has been established using synthetic DNA templates, with an observed LoD of 200 copies/reaction under the tested conditions. However, clinical utility must be validated using biological specimens. Future work will therefore involve testing the device with nasopharyngeal swabs collected from patients. This transition from synthetic templates to clinical matrices introduces additional variables, including variable sample input, RNA stability, and amplification inhibitors such as mucins, host proteins, salts, and blood-derived components that may delay amplification or reduce product yield (Nwe *et al.* 2024).

The validation protocol should involve a comparative study against the gold-standard RT-PCR method, with diagnostic sensitivity and specificity quantified to benchmark the device against established clinical standards. This stage will also require integration of an upstream sample-preparation workflow suitable for RNA viruses. Previous studies have demonstrated relevant approaches, including paper-based RNA extraction followed by in situ RT-LAMP and lateral-flow detection from nasopharyngeal specimens (Nguyen *et al.* 2023), and paper-origami devices integrating nucleic-acid extraction, LAMP, and detection from whole blood (Xu *et al.* 2016). These examples suggest that simple lysis and paper-based or elution-free nucleic-acid capture would be more compatible with the passive device architecture developed in this thesis than centrifugation- or pump-dependent purification workflows.

Lyophilisation and Storage

Currently, the device requires the manual loading of liquid LAMP reagents and running buffer immediately prior to use. To achieve a true Point-of-Care status, the reagent mix must be pre-loaded and shelf-stable without the need for cold storage or manual addition of liquid reagents. The short-term focus will be on the lyophilisation (freeze-drying) of the LAMP reagents directly onto the paper substrate within the reaction chamber.

Research in the field suggests that the inclusion of trehalose in lyophilised LAMP mixes significantly enhances the stability and enzymatic activity of the polymerase. Specifically, formulations utilising trehalose concentrations of 5-15% have been shown to yield optimal performance after ambient storage (Jothinarayanan *et al.* 2023). Long-term dry storage of enzyme-based isothermal amplification reagents with trehalose and dextran has demonstrated stability at room temperature for up to a year while retaining amplification capability (Kumar *et al.* 2020). We will investigate the use of these excipients, which stabilise the protein structure of the polymerase and reverse transcriptase during lyophilisation and storage (Åzay *et al.* 2021). The goal is to produce a dry-reagent device where the amplification mix is pre-dried in the reaction zone, which would simplify the user workflow to a single step: adding the sample buffer to rehydrate the reagents and initiate the reaction. Stability studies will be conducted to ensure the enzymes retain activity after storage at ambient temperatures for at least 3-6 months.

6.2.2 Long-Term Vision: System Evolution and Scalability

Manufacturing Scale-up

As discussed in the fabrication challenges of Chapter 3, the manual assembly and stencil printing methods used in this thesis are suitable for prototyping but not for mass production. To meet the global demand for low-cost diagnostics, the manufacturing process should transition to Roll-to-Roll (R2R) processing. R2R enables the high-speed printing and patterning of functional materials on a moving flexible web, drastically lowering per-unit production costs while significantly improving batch-to-batch reproducibility (H.-S. Kim *et al.* 2024). Moreover, R2R has been successfully applied to integrated microfluidic immunosensors and printed electronics, demonstrating the feasibility of continuous antibody dispensing, microfluidic channel replication, and sensor printing on flexible substrates (Liedert *et al.* 2020).

The primary long-term objective is to adapt the screen-printing protocols developed for the PTC heaters into a continuous R2R line. This involves optimising the rheology of the PTC and silver inks for high-speed rotary screen printing and developing automated lamination stations to bond the paper, adhesive, and plastic layers with micron-scale precision. Such process engineering will minimise the resistance variability observed in current manual prototypes and enable the consistent, large-scale production of flexible diagnostic devices.

Alternatively, if the direct scaling of the silver-PTC heaters proves economically challenging, we will explore the development of Zinc-based Interdigitated Electrodes (IDEs) as a sustainable solution. Building on the “Growth-and-Transfer” additive manufacturing process introduced in Section 3.1.11, this approach allows for the rapid production of biodegradable metallic tracks without toxic etchants. To integrate this at an industrial scale, a robust screen-printing system should be employed to deposit the viscous PTC layer over the transferred zinc electrodes.

The critical engineering challenge for this alternative is minimising the intrinsic series resistance of the zinc traces, which was identified as a limiting factor in earlier micro-scale designs. Future work will focus on optimising the geometric parameters by minimising the electrode gap spacing (to reduce the PTC resistance) while simultaneously increasing the trace thickness to improve conductivity. Additionally, the versatility of the growth-and-transfer method allows for the potential substitution of zinc with higher-conductivity materials, such as silver. By tuning these parameters, the optimised IDEs have the potential to achieve the goal temperatures at lower driving voltages compared to the current architecture, thereby enhancing energy efficiency for battery-powered operation.

Compact and Disposable Battery

A major limitation of the current prototype is its reliance on a rigid, external Lithium-ion battery. While effective for research, it adds weight, cost, and disposal concerns. A primary long-term goal, pursued in collaboration with Jeff Kettle’s group (electrode and circuit printing) and Guanchen Li’s group (battery design and optimisation), is the development of a disposable, integrable 2D battery. The vision is to print the battery directly onto a flexible substrate (either paper or thin plastic), allowing it to be laminated directly into the device structure.

The development of paper-based energy storage systems has emerged as a vibrant area of research, driven by the critical need for sustainable power sources in disposable electronics (Juqu *et al.* 2022). Unlike rigid coin cells, paper-based batteries utilise the cellulose matrix as both a flexible substrate and a functional separator, offering distinct advantages in terms of biodegradability, low-cost fabrication, and seamless integration with other paper-based components (Aryal *et al.* 2024). These devices typically integrate conductive inks (e.g., zinc, graphite, or carbon nanomaterials) to create safe, sustainable power sources (Laforgue *et al.* 2026). Although single paper-based cells typically achieve low voltages (e.g., 1.2–1.5 V for zinc-based chemistries), researchers have successfully demonstrated that stacking cells or connecting multiple batteries in series can scale the voltage to levels comparable with lithium-ion batteries (Y. Wang *et al.* 2023, Ishibashi *et al.* 2024).

Moreover, recent innovations include water-activated paper batteries that remain inert until a fluid electrolyte is introduced (Poulin *et al.* 2022). In this system, the dry paper separator is impregnated with salts (e.g., sodium chloride), which dissolve upon the addition of water or an aqueous sample to activate the ionic conductivity and initiate power generation. This “switch-like” activation mechanism is particularly advantageous for our proposed device, as it allows the heating process to be triggered automatically by the loading of the sample or running buffer. By stacking these fluid-activated cells or integrating them with lower-power consumption PTC heaters (as proposed in the previous section), we can achieve a fully self-powered, automated diagnostic platform where the addition of the liquid sample simultaneously initiates both the biochemical reaction and the thermal control system.

Encapsulation and Cassette Design

The current device uses a multi-layer adhesive film and PMMA assembly. The long-term vision is to encapsulate the paper-based microfluidics into a robust, injection-moulded or 3D-printed plastic cassette. This housing would provide mechanical protection, ensure consistent internal pressure, and prevent contamination.

The cassette design will aim to integrate a nucleic acid extraction module upstream of the reaction chamber and the battery-PTC heater system beneath the reaction chamber. By incorporating an origami paper structure (Reboud *et al.* 2019) or a lysis chamber preloaded with chemical lysis buffer (K. P. Jiang *et al.* 2024) to process the RNA extraction, the device could

perform lysis, washing, and elution steps through simple mechanical actions (e.g., series folding steps or button press). The final device would present a closed ecosystem with a single viewing window for the lateral flow strip, protecting the user from amplicon contamination and ensuring biosafety.

Smartphone Integration and Automated Readout

Finally, to eliminate the subjectivity of naked-eye interpretation (particularly for the complex 10-spot pattern of the multiplex array), we aim to develop a smartphone-based readout system to improve user-friendliness.

Future work will involve developing a dedicated mobile application that utilises the phone's camera and computer vision algorithms to capture the strip image, identify the control dots, and quantify the colour intensity of each target zone. This system could provide a binary “Positive/Negative” result for each pathogen, remove ambiguity, and enable connectivity for epidemiological reporting (Guo *et al.* 2021). This digital integration would transform the device from a simple test strip into a connected health tool.

Appendices

A Synthetic DNA sequences

The following table provides the full nucleotide sequences for the synthetic DNA templates used in this study for SARS-CoV-2, Influenza A, Influenza B, RSV A, and RSV B.

Table 7: Sequence for synthetic DNA templates

Target Name	Sequence (5' → 3')
SARS-COVID-2 (500bp)	TGAGTGGAGTATGGCTACATACTACTTATTTGATGAGT CTGGTGAGTTTAAATTGGCTTCACATATGTATTGTTCT TTCTACCCCTCCAGATGAGGATGAAGAAGAAGGTGATT GTGAAGAAGAAGAGTTTGAGCCATCAACTCAATATGA GTATGGTACTGAAGATGATTACCAAGGTAAACCTTTG GAATTTGGTGCCACTTCTGCTGCTCTTCAACCTGAAGA AGAGCAAGAAGAAGATTGGTTAGATGATGATAGTCAA CAAAGTGTGGTCAACAAGACGGCAGTGAGGACAATC AGACAAGTACTATTCAAACAATTGTTGAGGTTCAACCT CAATTAGAGATGGAAGTTACACCAGTTGTTTCAGACTA TTGAAGTGAATAGTTTTAGTGGTTATTTAAACTTACT GACAATGTATACATTAATAAATGCAGACATTGTGGAAG AAGCTAAAAAGGTAAAAACCAACAGTGGTTGTTAATGC AGCCAATGTTTACC
Influenza A (248bp)	CCCCTCAAAGCCGAGATCGCACAGAGACTTGAAGATG TCTTTGCTGGGAAGAACACCGATCTTGAGGCTCTCATG GAATGGCTAAAGACAAGACCGATCCTGTCACCTCTGA CTAAGGGGATTTTAGGATTTGTGTTACGCTCACCGTG CCCAGTGAGCGAGGACTGCAGCGTAGACGCTTTGTCC AAAATGCCCTTAATGGGAATGGGGATCCAAATAACAT GGACAGAGCAGTTAAACTGTATAG
Influenza B (237bp)	AACACAAATTGAGGTGGGTCCGGGAGCAACCAATGCC ACCATAAACTTCGAAGCAGGAATTCTGGAGTGCTATG AAAGACTTTCATGGCAAAGGGCCCTTGACTACCCTGG TCAAGACCGTCTAAACAGACTAAAGAGGAAATTAGAG TCAAGAATAAAGACCCACAACAAAAGTGAGCCTGAA AGTAAAAGGATGTCTCTTGAAGAGAGAAAAGCAATTG GAGTAAAAATGATGAA
RSV A (269bp)	TCACGAGGGCTCCACATACACAGCTGCTGTTCAATAC AATGTCCTAGAAAAAGACGATGATCCTGCATCACTTA CAATATGGGTGCCCATGTTCCAATCATCCATGCCAGCA GATTTACTTATAAAAGAACTAGCTAATGTCAACATACT AGTGAAACAAATATCCACACCCAAGGGACCTTCATTA AGAGTCATGATAAACTCAAGAAGTGACAGTGCTAGCAC AAATGCCCAGCAAATTTACCATATGTGCCAATGTGTCC TTGGATG
RSV B (242bp)	TATAGTAACAAAAAAGAACAAGATGGGGCAAATAT GGAAACATACGTGAACAAGCTTCACGAAGGCTCCACA TACACAGCAGCTGTTTCAGTACAATGTTCTAGAAAAAG ATGATGATCCTGCATCACTAACAATATGGGTGCCTATG TTCCAGTCATCTGTACCAGCAGACTTGCTCATAAAAGA ACTTGCAAGCATCAACATACTAGTGAAGCAGATCTCT ACGCCCAAAGGACCTTCAC

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