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A Foldable Thermoplastic Microdevice Integrating Isothermal Amplification and Schiff-Reaction-Based Colorimetric Assay for the Detection of Infectious Pathogens

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Abstract: In this study, we introduce a plastic-based foldable microdevice that integrates loop-mediated isothermal amplification (LAMP) and a colorimetric assay based on the Schiff reaction to detect the genes of infectious bacteria. The device comprises two sides: a sample zone containing amplification chambers and a detection zone for the colorimetric assay. The detection zone contains poly(methyl methacrylate) structures for transferring the colorimetric reagent-soaked glass micro-fiber paper into the sample chambers. Specific genes of *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pneumoniae* (*S. pneumoniae*), the most common bacterial infection causes, were amplified by LAMP assay. The *S. aureus* gene was detected up to 10 fg/μL and the *S. pneumoniae* gene up to 0.1 pg/μL. The amplified target genes were visually identified using a colorimetric assay with Schiff's reagent, which showed clear color discrimination through a reaction with aldehyde groups derived from the DNA in the amplicons. The introduced method, integrating amplification and detection processes in a single device, is expected to be utilized in point-of-care testing analysis for the simple and rapid detection of infectious pathogens.

Keywords: foldable microdevice; loop-mediated isothermal amplification (LAMP); Schiff reaction; colorimetric assay; point-of-care testing (POCT)

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1. Introduction

Various types of infectious diseases typically come from harmful bacteria such as *Staphylococcus* and *Streptococcus*, which invade the human body [1]. *Staphylococcus aureus* (*S. aureus*) is a Gram-positive bacterium that is a representative agent of many purulent diseases. *S. aureus* can infect almost all tissues in the human body, including osteoarticular, skin, and soft tissue [2–4]. *Streptococcus pneumoniae* (*S. pneumoniae*) is a Gram-positive bacterium that causes infections of the ear, respiratory tract, and joints. In particular, pneumolysin (ply) is a virulence factor of *S. pneumoniae* involved in pneumococcal diseases such as pneumonia, septic arthritis, and meningitis [5–7]. Therefore, rapid identification of the causative organisms is crucial for the early diagnosis and treatment of many bacterial diseases [8,9]. Because genetic testing identifies possible pathogenic bacteria based on their specific gene sequences, it can be used to identify the causative pathogens of several diseases [10].

The nucleic acid amplification test (NAAT), a technique that amplifies particular sequences of nucleic acids, has been used to detect viruses or pathogens. NAAT has been extensively applied in molecular diagnostics, as it can analyze results with a small sample size [11,12]. In general, NAAT involves the extraction, amplification, and detection steps of nucleic acids to be applied for molecular diagnosis. Among various amplification methods, polymerase chain reaction (PCR) is a well-known technology for molecular diagnostics with the advantages of high specificity and sensitivity [13]. PCR uses three thermal cycles to amplify nucleic acids, which limits point-of-care testing (POCT) applications. Therefore,

amplification methods under isothermal conditions, such as loop-mediated isothermal amplification (LAMP), transcription-mediated amplification (TMA), and recombinase polymerase amplification (RPA), have been developed as new POCT methods [14]. Among these techniques, LAMP can amplify nucleic acids with high specificity using four to six primers and strand-displacement DNA polymerases [15]. LAMP is widely used for POCT because of its rapidity and high sensitivity. The LAMP process can be completed within 1 h, with a sensitivity similar to PCR [16–20].

LAMP amplicons have been analyzed using various methods, such as turbidity, pH-dependent methods, fluorescence, and colorimetric assays [21–23]. In particular, the colorimetric assay is suitable for POCT because it does not require complicated equipment such as a fluorometer to analyze the results. In this study, we used fuchsin dye to visually detect LAMP amplicons using a colorimetric assay. Fuchsin is a natural dye that can be used for the biological staining of cytology samples [24]. According to a previous study, the reaction with sodium sulfite can decolorize the original magenta color of fuchsin dye by breaking the bond of the central C atom. It can also be used for DNA detection because decolored fuchsin restores its color due to structural changes when acid-hydrolyzed DNA binds to fuchsin [25,26]. Although this fuchsin-based method provides a simple way to analyze DNA in LAMP amplicons, an additional step is required to decolorize the fuchsin. To simplify the detection steps, we used Schiff's reagent, which is a colorless product synthesized using basic fuchsin and sodium sulfite [27]. Schiff's reagent is colorless; however, it can react with the aldehyde groups produced by the acid hydrolysis of LAMP amplicons and turn purple. The aldehyde groups can be produced when DNA is hydrolyzed under acidic conditions, thus the color of the final reaction product will turn purple if it contains the LAMP amplicons. Color discrimination derived from the reaction between Schiff's reagent and the aldehyde groups of DNA can be easily confirmed with the naked eye [28].

Here, we introduce a plastic-based foldable microdevice that integrates LAMP amplification with a colorimetric assay using Schiff's reagent for the early diagnosis of infectious diseases. This device has two sides: a sample zone containing amplification chambers and a detection zone containing colorimetric reagent-soaked paper. The detection zone contains the PMMA-based cylindrical structure for attaching the paper soaked with colorimetric reagent. Using this device, specific genes of *S. aureus* and *S. pneumoniae* were amplified by LAMP within 45 min. After performing the amplification using the device, the LAMP amplicons were identified using a Schiff's reagent-based colorimetric assay. The acidic conditions produced by HCl can hydrolyze DNA to produce aldehyde groups, which can bind to the sulfonate groups in Schiff's reagent. The Schiff's reagent, which was originally colorless, turned purple when it reacted with the aldehyde groups of the DNA in the LAMP amplicons. When applying the colorimetric assay to the microdevice, the HCl was soaked in glass microfiber paper and completely dried. Thus, this method provides more safety compared to those used in previous studies [28]. In this study, we developed a foldable microdevice that can be used to simply detect infectious pathogens. Using this device, the amplification of DNA by the LAMP method and intuitive monitoring of the results using a colorimetric assay can facilitate the detection of pathogens. In addition, the integration of LAMP and colorimetric assays enables all steps to be performed on a single device, allowing faster detection by reducing the necessity of performing each step separately. This integrated device can be easily applied to limited resource situations because it does not require an additional analysis machine. Therefore, the introduced device provides effective and time-saving detection methods with the potential to be used for diagnosing several diseases in the clinical field, where on-site diagnosis becomes urgent.

2. Materials and Methods

2.1. Chemicals and Materials

Basic fuchsin, sodium sulfite (Na_2SO_3), and activated charcoal were purchased from Sigma-Aldrich (St. Louis, MO, USA). A 1 M hydrochloric acid (HCl) solution and a 9% sodium hypochlorite (NaOCl) solution were obtained from Daejeong (Siheung, Republic

of Korea). LAMP reagents consisting of a 10× buffer solution, 100 mM $MgSO_4$, and *Bst* DNA polymerase were obtained from New England Biolabs (Ipswich, MA, USA). dNTPs (10 mM), syringe filters, and sealing films were obtained from Bio-FACT (Daejeon, Republic of Korea). A 100 bp DNA size marker was obtained from DyneBio (Seongnam, Republic of Korea). Loading dye was purchased from WizBio-Solutions (Seongnam, Republic of Korea). Universal pH indicator paper (pH 1–11) was obtained from HYUNDAIMICRO (Anseong, Republic of Korea), and pH test paper (pH 1.4–3.0, pH 8.0–9.6) was obtained from ADVANTEC (Tokyo, Japan). Agarose powder was obtained from BioShop (Burlington, ON, Canada). Poly(methylmethacrylate) (PMMA) (3 mm-thick) was obtained from Goodfellow (Coraopolis, PA, USA). Double-sided tape was obtained from 3MTM (St. Paul, MN, USA). Glass microfiber filter paper was obtained from CHMLAB (Barcelona, Spain).

2.2. LAMP Amplification

For the DNA templates of *S. aureus* and *S. pneumoniae*, partial sequences of the *kat*A and *ply* genes were synthesized and cloned into the plasmid using Cosmo Genetech (Seoul, Republic of Korea). The LAMP primer sets used to amplify the *S. aureus* plasmid and *S. pneumoniae* plasmid were designed using Primer Explorer V5 software and synthesized by Cosmogenetech (Seoul, Republic of Korea); their sequences are shown in Table 1. The reaction mixture for the LAMP assay consisted of 1.2 mM of dNTPs, 6 mM of $MgSO_4$, 10×buffer solution, 1.6 µM of FIP and BIP primers, 0.2 µM of F3 and B3 primers, 0.8 µM of LF primer and 4 units of *Bst* polymerase, and 1 µL of the target DNA templates (1 ng/µL). To ensure the accuracy of the results, a negative sample that did not contain DNA was used. After thoroughly adding the reagents with DNA, the LAMP assay was operated at 65 °C for 45 min to amplify the target DNA. Gel electrophoresis was used to analyze the LAMP results. These results were further analyzed using a Schiff's reagent-based colorimetric assay.

Table 1. Primer sequences used to amplify plasmids containing *S. aureus* and *S. pneumoniae*.

Target	Primer Name	Primer Sequences (5′-3′)
*kat*A gene (*S. aureus*)	F3	AGATCTTAATGTCAGATAGAGG
	B3	TTGGATGAATCGCGATCT
	FIP	ACACGTTCACCAGAATCATTATACAG ATTCCTAAAGATTTGCGTCAC
	BIP	AATTCCATTTTAGAACGCAACAAGG TGCTATAATTTCAGCAGCTACT
	LB	GTGTGTGAACCGAACCCATGCA
ply gene (*S. pneumoniae*)	F3	AAAGAAGCGGAGCTGTC
	B3	TCCACTTGGAGAAAGCTATC
	FIP	ACTACGAGAAGTGCTCCAGGTGATAT TTCTGTAACAGCTACCAA
	BIP	AATCCCACTCTTCTTGCGGTGCTACTT GCCAAACCAGG
	LB	CGATCGTGCTCCGATGACTT

2.3. Colorimetric Detection Using Schiff's Reagent

To obtain Schiff's reagent for the colorimetric assay, 50 mg of fuchsin was added to 9 mL of boiling water. When fuchsin was fully dissolved in water and the solution was cooled to approximately 50 °C, 1 mL of 1M HCl was slowly added to the solution. Subsequently, 100 mg of Na_2SO_3 was added, and the mixture was stirred for 5 min using a magnetic stirrer. After mixing, the solution was wrapped in aluminum foil and kept at room temperature overnight. Subsequently, 50 mg of activated charcoal was added to the solution and filtered through a syringe filter to remove impurities. Finally, Schiff's reagent was placed in a glass bottle and stored at 4 °C to prevent denaturation. After LAMP amplification, the DNA was hydrolyzed under acidic conditions by heating the LAMP amplicons with HCl for 10 min at a constant temperature. The chemical interaction

between the aldehyde groups formed from DNA and Schiff's reagent is shown in purple, and the absorbance values were analyzed using an Epoch™ spectrophotometer (BioTek Instruments, Winooski, VT, USA). Figure 1 shows the overall principles of the colorimetric assay using Schiff's reagent.

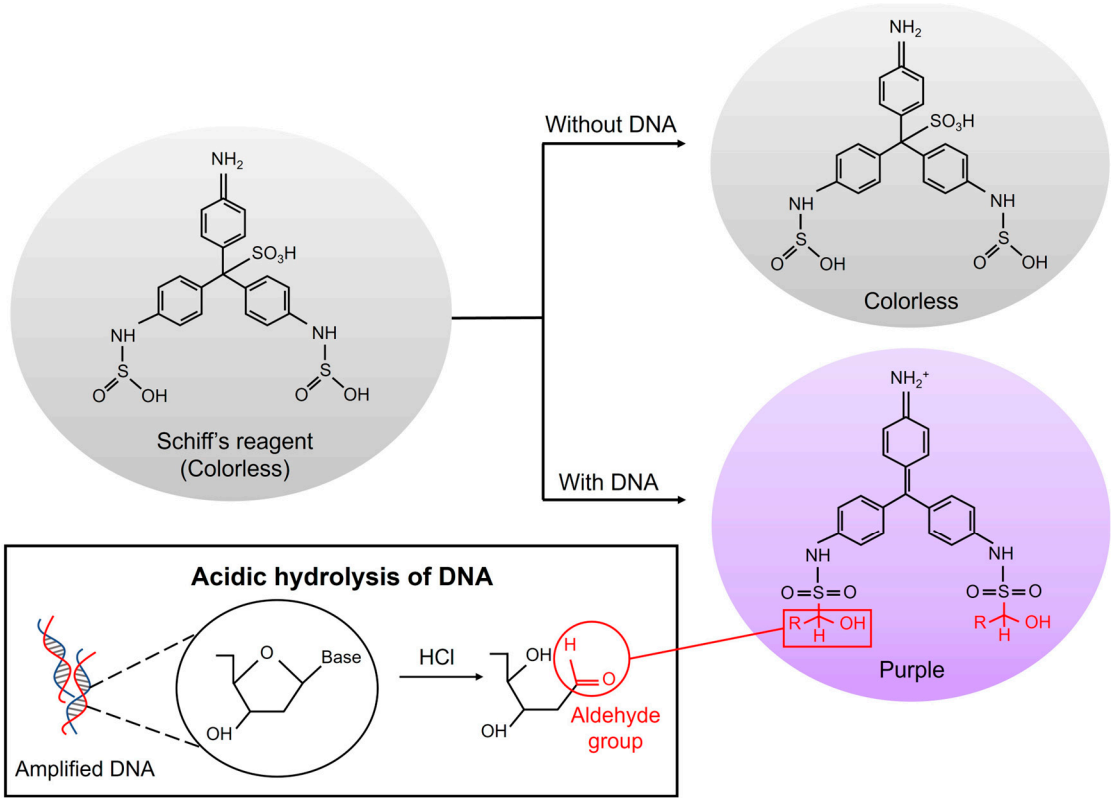


Figure 1. Illustration showing the colorimetric assay strategy using Schiff's reagent.

2.4. Fabrication and Operation of a Foldable Microdevice

A foldable microdevice was designed to simplify the amplification and detection processes. The structure and mechanism of the microdevice are shown in Figure 2. The device was constructed with two sides: a sample zone containing amplification chambers and a detection zone for performing a colorimetric assay. The two sides of the microdevice were made of PMMA (3 mm thickness) and engraved using a plastic processing machine. The sample zone contained four amplification chambers (5 mm in diameter, 2.7 mm in depth, and 40 µL in volume), and the detection zone contained four cylindrical PMMA features (4.5 mm in diameter, 2.5 mm in depth). The HCl-soaked glass microfibers were attached to the cylindrical PMMA features using double-sided tape. In this step, HCl-soaked glass microfiber paper was applied to the microdevice after complete drying. The LAMP reagents and target DNA were deposited into the amplification chambers and treated at 65 °C for 45 min to amplify the DNA. A sealing film was used to close the amplification chambers and minimize evaporation during amplification. After amplification, the sealing film was removed, and the HCl-soaked glass microfiber in the detection zone was folded to physically contact the LAMP samples in the amplification chambers. After 1 min to release HCl, the detection zone was unfolded and treated at 65 °C for 10 min to hydrolyze the LAMP amplicons. A sealing film was used to prevent evaporation during acid hydrolysis. After the acid hydrolysis was completed, 20 µL of Schiff's reagent was added to the chamber, obtaining acid-hydrolyzed LAMP samples, which induced the colorimetric reaction of acid-hydrolyzed LAMP amplicons in the amplification chambers. Twenty microliters of Schiff's reagent were added; this amount is sufficiently small to cause some safety problems. Additionally, the microdevice was cleaned using a 0.3% NaOCl solution to prevent sample

contamination [29]. Figure 2 shows schematics of the overall construction and operation process of the foldable microdevice.

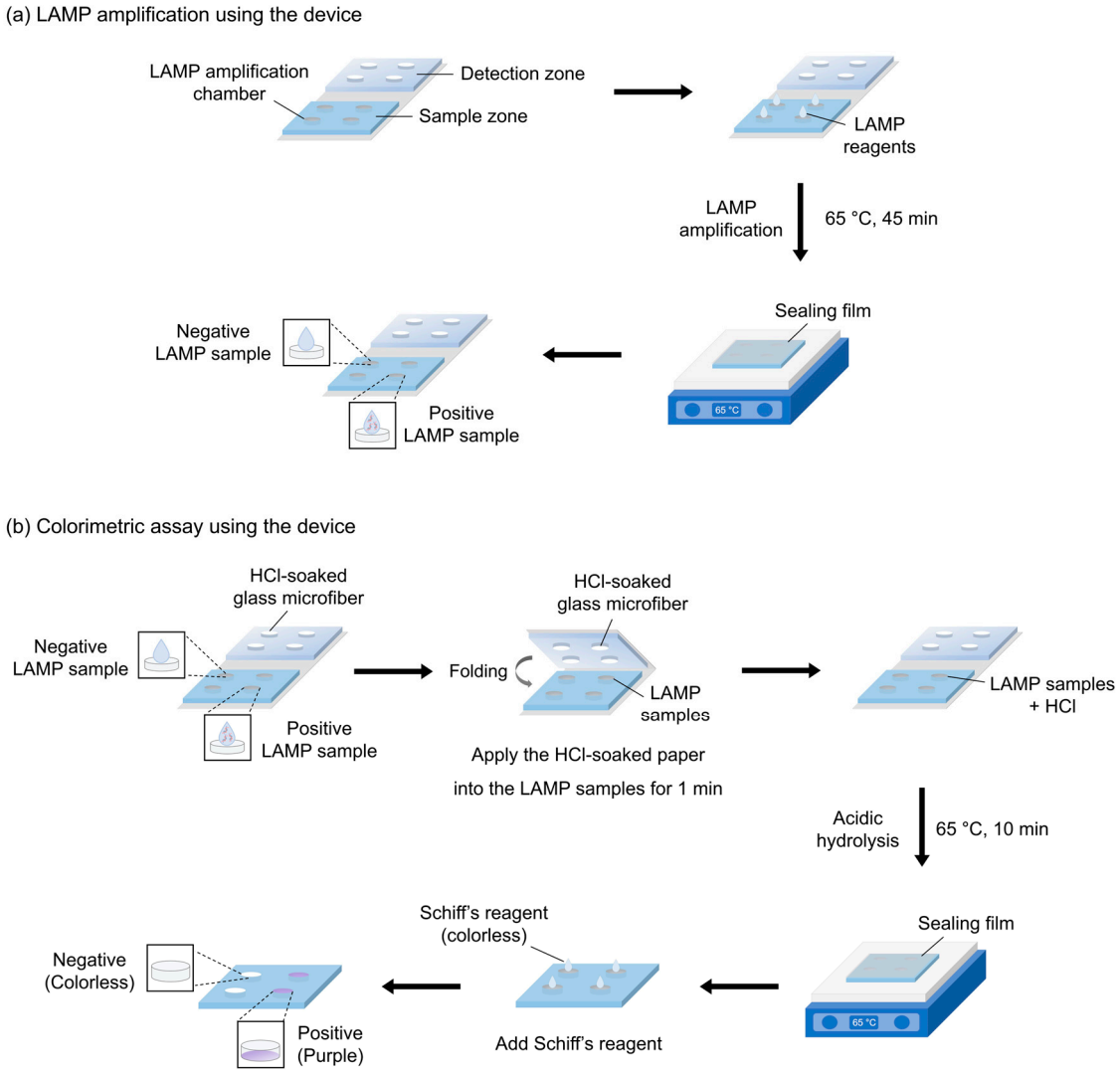


Figure 2. Illustration showing the overall operation process of the foldable microdevice. (**a**) Process of LAMP amplification and (**b**) colorimetric assay using the device.

3. Results and Discussions

3.1. Acid Hydrolysis of DNA in Amplicons

The color change depends on the structural changes in the sulfonate groups in Schiff's reagent, derived from the binding of the aldehyde groups produced when DNA is hydrolyzed under acidic conditions. Therefore, HCl was used to hydrolyze the DNA of the LAMP amplicons by converting the ribose ring of the DNA into aldehyde groups. For the positive LAMP samples, the colorless Schiff's reagent turned purple, whereas the negative LAMP samples remained colorless. Figure 3 shows the effect of the HCl concentration on the colorimetric assay using Schiff's reagent. The results of the colorimetric assay were analyzed using a spectrophotometer by comparing absorbance values. The *S. aureus* amplicons with Schiff's reagent showed an absorption peak at 570 nm (Figure 3a), and the absorbance value of the resulting Schiff's reagent color was highest when 80 mM HCl was used (Figure 3b). Similarly, the *S. pneumoniae* amplicons showed an absorption peak at 570 nm (Figure 3c), and the absorbance value of the resulting Schiff's reagent color was the highest when 100 mM HCl was used (Figure 3d). Regardless of the type of target DNA tested, the absorbance value of the resulting color was highest when 80–100 mM HCl was

used. In this study, 100 mM was selected as the optimal concentration for the multiplex detection of *S. aureus* and *S. pneumoniae* because, for both targets, 100 mM HCl displayed the highest absorbance values. The color change phenomenon was also evaluated based on hydrolysis time. More than 10 min of hydrolysis was required to display distinguishable colors between the negative and positive samples. Based on these results, 10 min was selected as the optimal hydrolysis reaction time (Figure 4).

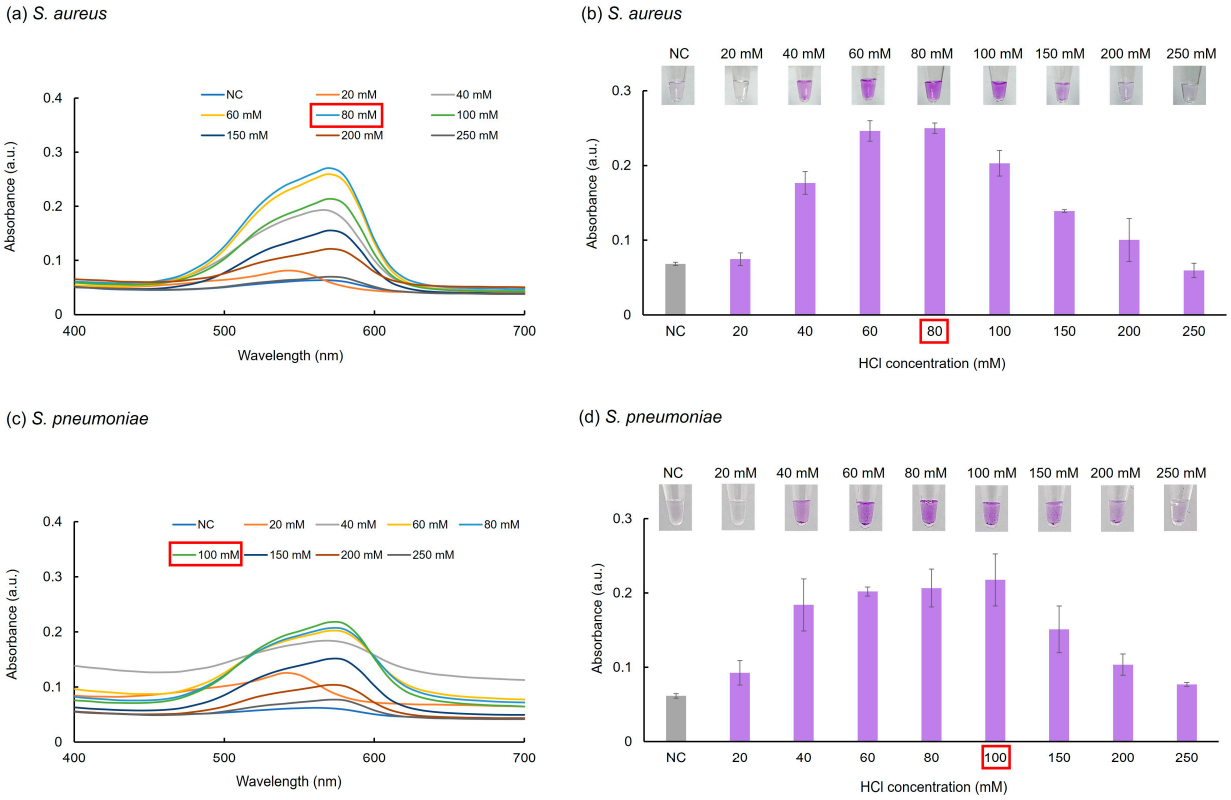


Figure 3. Results indicating the effect of HCl concentration on the acid hydrolysis of the LAMP amplicons. Images showing the absorbance spectra of the colorimetric assay results with different concentrations of HCl for (**a**) *S. aureus* and (**c**) *S. pneumoniae*. Colorimetric assay results and absorption graphs with different concentrations of HCl for (**b**) *S. aureus* and (**d**) *S. pneumoniae*.

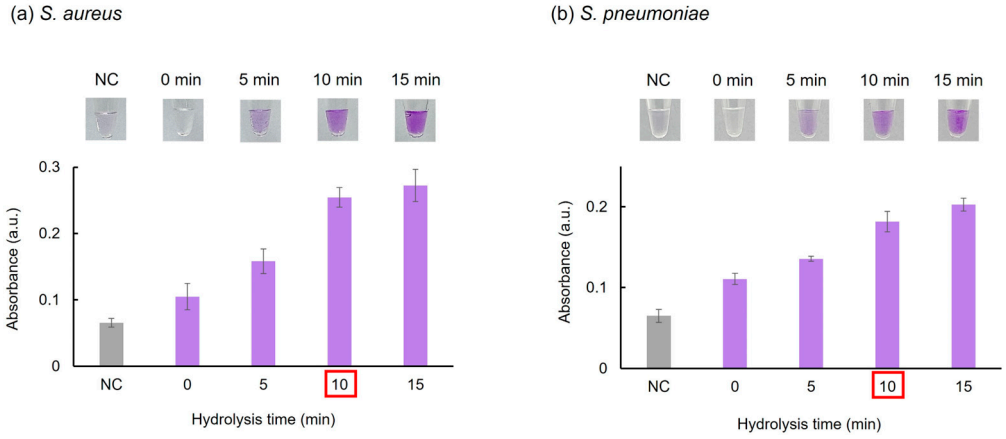


Figure 4. Results indicating the effect of reaction time on the acid hydrolysis. Colorimetric assay results and absorption graphs at varying times of acid hydrolysis for (**a**) *S. aureus* and (**b**) *S. pneumoniae*.

3.2. Specificity Test of the LAMP Assay

Figure 5 shows the specificity of the LAMP assay performed using the *S. aureus* and *S. pneumoniae* primer sets. By using the *S. aureus* primer sets, the *S. aureus* plasmid was successfully amplified; however, the *S. pneumoniae* plasmid was not. Similarly, using the *S. pneumoniae* primer sets, the *S. pneumoniae* plasmid was successfully amplified, while the *S. aureus* plasmid was not. The results were analyzed by electrophoresis and a colorimetric assay with Schiff's reagent (Figure 5). Similar to the gel electrophoresis results, the *S. aureus* plasmids amplified using the *S. aureus* primer sets, and the *S. pneumoniae* plasmids amplified using the *S. pneumoniae* primer sets displayed high absorbance values. In contrast, plasmids with mismatched primer sets displayed absorbance values similar to those of the negative control.

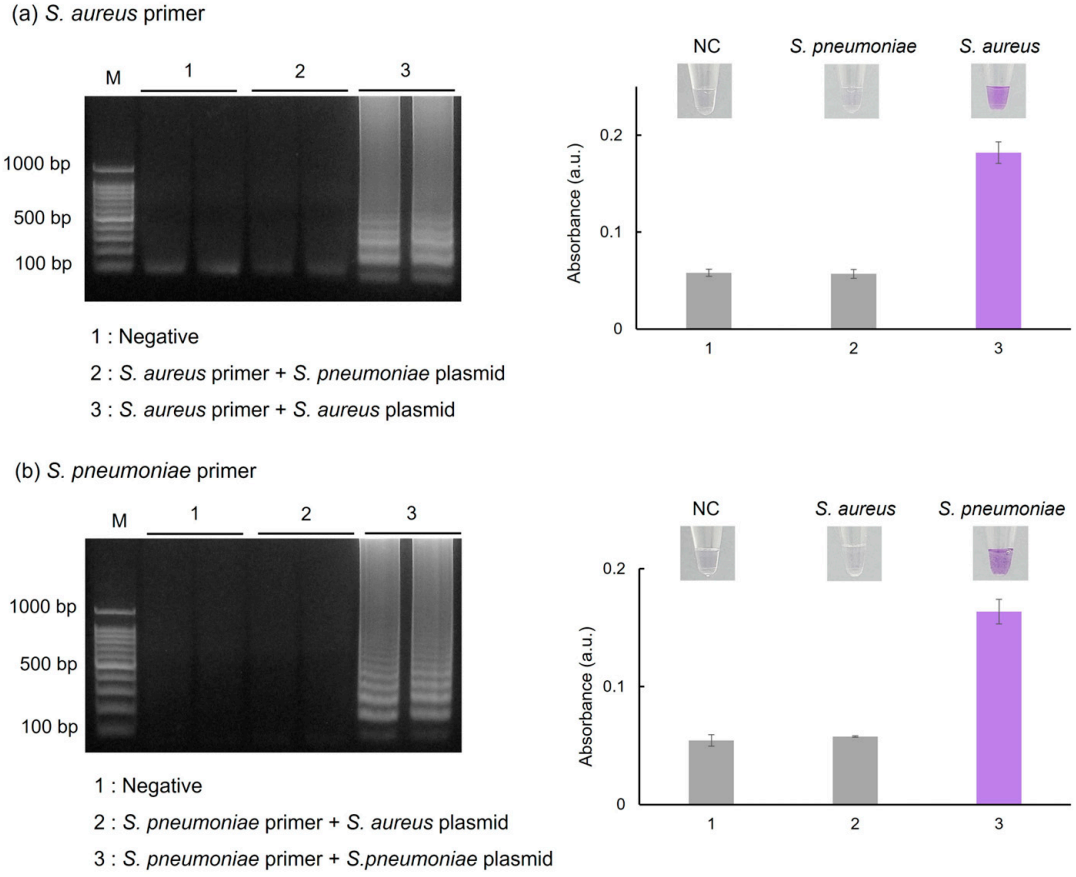


Figure 5. Results analyzed by gel electrophoresis and colorimetric assay when a specificity test was performed using (**a**) *S. aureus* primer sets and (**b**) *S. pneumoniae* primer sets.

3.3. Sensitivity Test of the LAMP Assay

Figure 6 shows the sensitivity of LAMP amplification. *S. aureus* and *S. pneumoniae* plasmids were diluted from 1 ng/μL to 10 fg/μL using a sequential dilution method. As shown in the result of gel electrophoresis (Figure 6a), the ladder-shaped band indicating the amplification of DNA was observed up to 0.1 pg/μL for *S. aureus* plasmids, which means that the limit of detection (LOD) was 0.1 pg/μL for *S. aureus* plasmids. The results of the colorimetric reaction also showed a similar pattern to the result of gel electrophoresis, exhibiting a purple color up to 1 pg/μL and remaining colorless below this concentration. The colorimetric results were obtained from the absorbance values. Similarly, the ladder-shaped bands appeared up to a concentration of 1 pg/μL for *S. pneumoniae* plasmids, which means that the LOD of *S. pneumoniae* plasmids was approximately 1 pg/μL. The colorimetric reaction exhibited a pattern similar to that of gel electrophoresis.

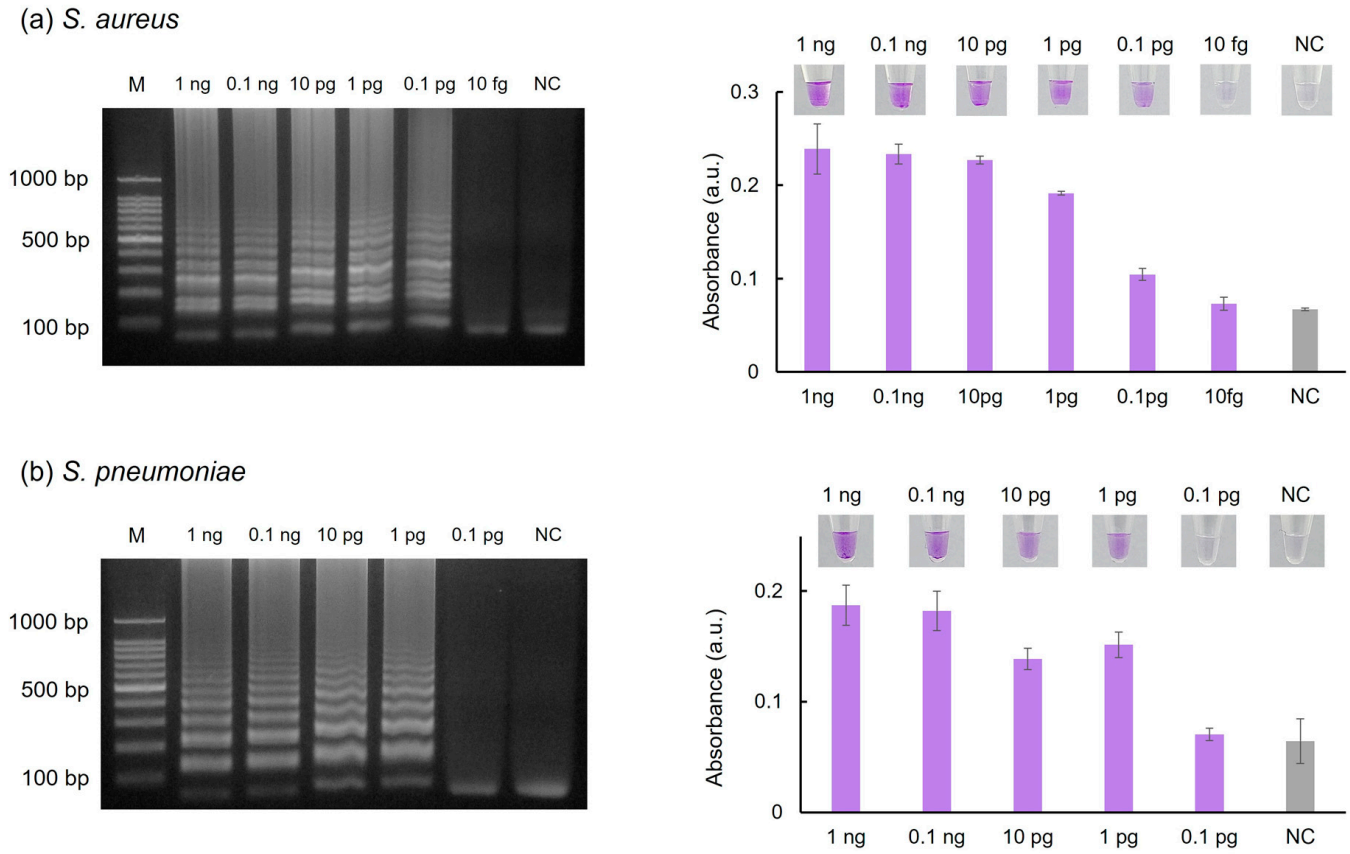


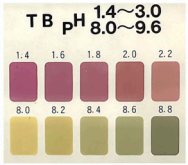
Figure 6. Sensitivity test of the LAMP assay. (**a**) Results of the gel electrophoresis and colorimetric assay of *S. aureus* plasmids with concentrations of 1 ng/μL to 10 fg/μL. (**b**) Results of the gel electrophoresis and colorimetric assay of *S. pneumoniae* plasmids with concentrations of 1 ng/μL to 0.1 pg/μL.

3.4. Effect of dNTPs on the Colorimetric Assay

When DNA is exposed to acidic conditions, purine bases, such as adenine and guanine, detach, producing free aldehyde groups. These aldehyde groups can chemically bind to Schiff's reagent to produce a purple color. To demonstrate the effect of dNTP concentration on the LAMP colorimetric assay, we prepared different concentrations of the dNTP solution (Figure 7a). Initially, 0.5 M HCl was added to form an acidic solution for the hydrolysis of dNTPs, and the resulting pH of the dNTPs solution was analyzed using two types of pH paper. Indeed, the dNTPs solution exhibited a pH in the range of 8–9, displaying a light pink color when reacted with Schiff's reagent because acid hydrolysis did not occur. However, the dNTPs that reacted with HCl exhibited a pH in the range of 1–2, displaying a purple color due to the formation of the aldehyde group that reacted with Schiff's reagent during heating. The color of the solution varied depending on the dNTP concentration. Significantly, when the concentration of dNTPs was 3 mM or higher, an intense color developed in which the dNTPs solution turned purple to a degree readily distinguishable by the naked eye. The concentration of dNTPs typically used in LAMP is approximately 1.2 mM. Based on this evidence, the dNTPs in the LAMP assay did not participate in the color development process (Figure 7a). At this concentration, the color of the dNTPs solution that reacted with the Schiff's reagent remained almost unchanged, displaying a color similar to that of the 0 mM dNTPs solution.

(a)

pH paper (1)



pH paper (2)

	Acidic hydrolysis using 0.5 M HCl								No acidic hydrolysis
Concentration of dNTPs (mM)	0	0.5	1.2	2	3	4	5	7	10	10
Colorimetric reaction										
pH paper (1)										
pH paper (2)										

(b)

Incubation / Acidic hydrolysis	1 min	5 min	10 min	5 hours	1 day
5 min	N P	N P	N P	N P	N P
10 min	N P	N P	N P	N P	N P

Figure 7. Effect of dNTPs on the colorimetric reaction using Schiff's reagent. (**a**) Results of the colorimetric assay using different concentrations of dNTPs. (**b**) Colorimetric reactions of LAMP samples by adding HCl and Schiff's reagent at the same time. N: Negative sample; P: Positive sample.

Figure 7b shows the color change in the LAMP samples after the reaction with HCl and Schiff's reagent at the same time. For positive samples containing LAMP amplicons, the purple color was retained for 1 d. However, the negative samples initially turned light pink and disappeared within 5 min. dNTPs with molecular weights lower than those of DNA are expected to also be able to produce aldehyde groups. Because of the instability of the aldehyde group, the pink color was rapidly lost in the negative sample. Therefore, we can conclude that in the negative sample, which also contained dNTPs and primers, the color of the sample remained colorless owing to two reasons: the lower amount of dNTPs used in the reaction and the rapid oxidization of the aldehyde group in the negative sample. Therefore, only the positive sample turned purple, whereas the negative sample remained almost colorless (Figure 7b).

3.5. Effect of the HCl-Soaked Paper on the Colorimetric Assay

To simplify the operation of the microdevice, glass microfiber filter paper was soaked with HCl, and the release pattern of HCl from the paper into the LAMP amplicon was observed. Figure 8a shows the results of the colorimetric assay when different concentrations of HCl-soaked glass microfiber filter papers were used. The concentration of the prepared HCl solution ranged from 20 to 250 mM, and the samples were soaked in a glass microfiber filter paper (4 mm in diameter) for 5 min. After fully drying, it was added to the LAMP amplicons to induce the acid hydrolysis of DNA. Figure 8b shows the intensities of the colors displayed on the paper when the LAMP samples reacted with the Schiff reagent and were analyzed using ImageJ software. A high color intensity was observed in the positive sample at a concentration of 100 mM HCl, which was clearly differentiated from the negative sample. In addition, the effect of HCl-soaked paper, which was used to produce an acidic condition in the DNA, was monitored using the change in pH. Figure 8c confirms that the pH of the LAMP amplicon solution significantly decreased within 1 min when reacted with HCl-soaked paper. From this, it was confirmed that HCl, which was

absorbed into the glass microfiber paper, could be released and induce acid hydrolysis of DNA in LAMP amplicons.

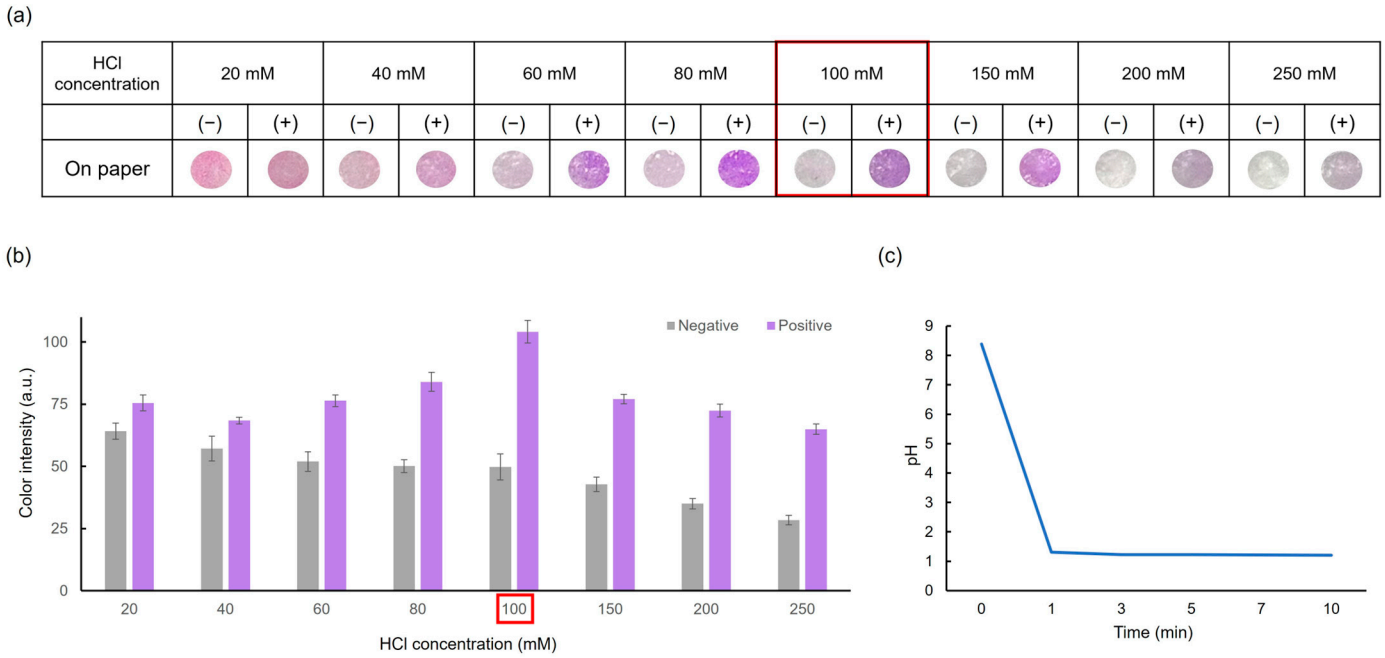


Figure 8. Results showing the effect of the HCl-soaked paper on the acid hydrolysis of LAMP amplicons at varying HCl concentrations (**a**) Results showing the color change in paper when different concentrations of HCl were applied. (**b**) Color intensity graph describing the colorimetric assay results of (**a**). (**c**) Time-dependent pH changes in the LAMP amplicons when HCl-soaked paper was added.

3.6. Visual Detection of Amplicons Using a Foldable Microdevice

Figure 9a shows the structure of the foldable microdevice. The microdevice comprises two parts: a sample zone and a detection zone. The detection zone can be folded toward the sample zone, allowing the colorimetric reagents to be easily transferred. To simplify the steps for the acid hydrolysis of DNA, HCl was soaked in glass microfiber paper and applied to the device. The HCl-soaked paper was fully dried and attached to the cylindrical PMMA features using double-sided tape. Folding the device allows the PMMA features to contact the amplification chambers in the sample zone, causing the HCl-soaked glass microfibers to react with the LAMP samples contained in the amplification chambers. Figure 9b shows the results of the LAMP amplification using the microdevice. LAMP reagents were deposited in amplification chambers and treated at 65 °C for 45 min. Since the ladder-shaped band is observed only in the positive sample, it can be confirmed that the amplification process was successfully performed on the device (Figure 9b). Figure 9c shows the results of colorimetric assays performed using the microdevice. After the acidic hydrolysis step using HCl-soaked glass microfiber, the colorimetric reaction was induced by adding Schiff's reagent. When the colorimetric reaction was complete, the chambers containing the positive sample turned purple, whereas those containing the negative sample remained colorless. The results were also analyzed by the intensity of color using the ImageJ software (Figure 9d).

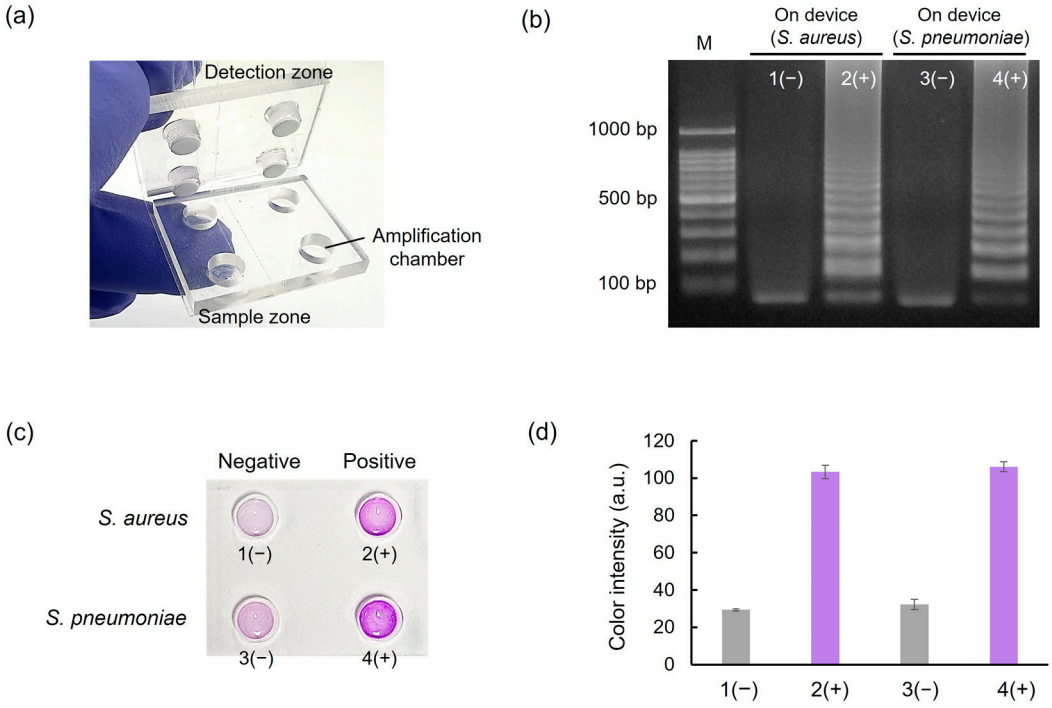


Figure 9. LAMP amplification and colorimetric assay performed using the foldable microdevice. (**a**) Photos showing the structure of the foldable microdevice. (**b**) Results of the agarose gel electrophoresis of the LAMP amplification performed using the microdevice. (**c**) Results of the colorimetric assay performed using the microdevice, and (**d**) analysis results using a color intensity graph.

4. Conclusions

In this study, we developed a plastic-based foldable microdevice for the facile identification of infectious disease-causing organisms. Using this device, *S. aureus* and *S. pneumoniae*, the most common infectious disease agents, were successfully amplified, and the results were confirmed by a colorimetric reaction. Rapid and sensitive amplification of the target DNA using LAMP enables target detection even with very small sample sizes. In addition, the detection process, based on a colorimetric assay using Schiff's reagent, contributed to intuitive visual detection without the use of any instruments for analysis. The use of glass microfiber filter paper to store the colorimetric reagents eliminated the need to add the reagents separately, contributing to the simple operation of the microdevice and making it suitable for POCT. The operation of the device does not require complicated equipment, such as a thermal cycler or centrifuge; thus, it provides a useful detection platform, especially in resource-limited environments. It can also be used to detect infectious agents in several diseases, contributing to their rapid diagnosis and treatment. Thus, the introduced device can pave the way for the development of a simple diagnostic tool that can be effectively used when immediate and urgent initial screening is needed. Furthermore, the introduced microdevice can provide better portability and accuracy through integration with technologies, such as smartphone analysis applications, with the potential to solve challenges in future public health.

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Data Availability Statement: The data presented in this study are available from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. McNeil, J.C.; Flores, A.R.; Kaplan, S.L.; Hulten, K.G. The indirect impact of the SARS-CoV-2 pandemic on invasive group A *Streptococcus*, *Streptococcus pneumoniae* and *Staphylococcus aureus* infections in Houston area children. *Pediatr. Infect. Dis.* **2021**, *40*, e313. [CrossRef] [PubMed]
2. Simon, C.; Rachel, T.; Benoît, L.G.; Emmanuel, H.; Denis, M.; Camille, L.; Estelle, L.P.; Laurie, C.; Helene, C.; Raphael, L.; et al. Septic arthritis of the facet joint is also a severe vertebral infection: A multicenter retrospective study of 65 patients. *Jt. Bone Spine* **2024**, *91*, 105703. [CrossRef]
3. McNeil, J.C.; Sommer, L.M.; Vallejo, J.G.; Boyle, M.; Hulten, K.G.; Kaplan, S.L.; Fritz, S.A. Going back in time: Increasing penicillin susceptibility among methicillin-susceptible *Staphylococcus aureus* osteoarticular infections in children. *Antimicrob. Agents Chemother.* **2023**, *67*, e01196-22. [CrossRef]
4. Howden, B.P.; Giulieri, S.G.; Lung, T.W.F.; Baines, S.L.; Sharkey, L.K.; Lee, J.Y.; Hachani, A.; Monk, I.R.; Stinear, T.P. *Staphylococcus aureus* host interactions and adaptation. *Nat. Rev. Microbiol.* **2023**, *21*, 380–395. [CrossRef] [PubMed]
5. Kim, J.; Lee, H.W.; Rhee, D.K.; Paton, J.C.; Pyo, S. Pneumolysin-induced autophagy contributes to inhibition of osteoblast differentiation through downregulation of Sp1 in human osteosarcoma cells. *Biochim. Biophys. Acta (BBA) Gen. Subj.* **2017**, *1861*, 2663–2673. [CrossRef]
6. Oh, M.W.; Lin, J.; Chong, S.Y.; Lew, S.Q.; Alam, T.; Lau, G.W. Time-resolved RNA-seq analysis to unravel the in vivo competence induction by *Streptococcus pneumoniae* during pneumonia-derived sepsis. *Microbiol. Spectr.* **2024**, *12*, e03050-23. [CrossRef]
7. Jacques, L.C.; Green, A.E.; Barton, T.E.; Baltazar, M.; Aleksandrowicz, J.; Xu, R.; Trochu, E.; Kadioglu, A.; Neill, D.R. Influence of *Streptococcus pneumoniae* within-strain population diversity on virulence and pathogenesis. *Microbiol. Spectr.* **2023**, *11*, e03103-22. [CrossRef]
8. Foddai, A.C.; Grant, I.R. Methods for detection of viable foodborne pathogens: Current state-of-art and future prospects. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 4281–4288. [CrossRef]
9. Riu, J.; Giussani, B. Electrochemical biosensors for the detection of pathogenic bacteria in food. *TrAC Trends Anal. Chem.* **2020**, *126*, 115863. [CrossRef]
10. Wang, C.; Liu, M.; Wang, Z.; Li, S.; Deng, Y.; He, N. Point-of-care diagnostics for infectious diseases: From methods to devices. *Nano Today* **2021**, *37*, 101092. [CrossRef]
11. Craw, P.; Balachandran, W. Isothermal nucleic acid amplification technologies for point-of-care diagnostics: A critical review. *Lab Chip* **2012**, *12*, 2469–2486. [CrossRef] [PubMed]
12. Obande, G.A.; Banga Singh, K.K. Current and future perspectives on isothermal nucleic acid amplification technologies for diagnosing infections. *Infect. Drug Resist.* **2020**, *13*, 455–483. [CrossRef]
13. Zhu, H.; Zhang, H.; Xu, Y.; Laššáková, S.; Korabečná, M.; Neužil, P. PCR past, present and future. *Biotechniques* **2020**, *69*, 317–325. [CrossRef] [PubMed]
14. Li, J.; Macdonald, J. Advances in isothermal amplification: Novel strategies inspired by biological processes. *Biosens. Bioelectron.* **2015**, *64*, 196–211. [CrossRef] [PubMed]
15. Notomi, T.; Okayama, H.; Masubuchi, H.; Yonekawa, T.; Watanabe, K.; Amino, N.; Hase, T. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.* **2000**, *28*, e63. [CrossRef] [PubMed]
16. Notomi, T.; Mori, Y.; Tomita, N.; Kanda, H. Loop-mediated isothermal amplification (LAMP): Principle, features, and future prospects. *J. Microbiol.* **2015**, *53*, 1–5. [CrossRef]
17. Augustine, R.; Hasan, A.; Das, S.; Ahmed, R.; Mori, Y.; Notomi, T.; Kevadiya, B.D.; Thakor, A.S. Loop-mediated isothermal amplification (LAMP): A rapid, sensitive, specific, and cost-effective point-of-care test for coronaviruses in the context of COVID-19 pandemic. *Biology* **2020**, *9*, 182. [CrossRef] [PubMed]
18. Kashir, J.; Yaqinuddin, A. Loop mediated isothermal amplification (LAMP) assays as a rapid diagnostic for COVID-19. *Med. Hypotheses* **2020**, *141*, 109786. [CrossRef] [PubMed]
19. Garg, N.; Ahmad, F.J.; Kar, S. Recent advances in loop-mediated isothermal amplification (LAMP) for rapid and efficient detection of pathogens. *Curr. Res. Microb. Sci.* **2022**, *3*, 100120. [CrossRef]
20. Li, Y.; Fan, P.; Zhou, S.; Zhang, L. Loop-mediated isothermal amplification (LAMP): A novel rapid detection platform for pathogens. *Microb. Pathog.* **2017**, *107*, 54–61. [CrossRef]
21. Alves, P.A.; de Oliveira, E.G.; Franco-Luiz, A.P.M.; Almeida, L.T.; Gonçalves, A.B.; Borges, I.A.; Rocha, F.d.S.; Rocha, R.P.; Bezerra, M.F.; Miranda, P.; et al. Optimization and clinical validation of colorimetric reverse transcription loop-mediated isothermal amplification, a fast, highly sensitive and specific COVID-19 molecular diagnostic tool that is robust to detect SARS-CoV-2 variants of concern. *Front. Microbiol.* **2021**, *12*, 713713. [CrossRef]

22. Fischbach, J.; Xander, N.C.; Frohme, M.; Glökler, J.F. Shining a light on LAMP assays' A comparison of LAMP visualization methods including the novel use of berberine. *Biotechniques* **2015**, *58*, 189–194. [CrossRef]
23. Anupama, K.P.; Nayak, A.; Karunasagar, I.; Maiti, B. Rapid visual detection of Vibrio parahaemolyticus in seafood samples by loop-mediated isothermal amplification with hydroxynaphthol blue dye. *World J. Microbiol. Biotechnol.* **2020**, *36*, 1–10. [CrossRef]
24. Bruneval, P.; Choucair, J.; Paraf, F.; Casalta, J.P.; Raoult, D.; Scherchen, F.; Mainardi, J.L. Detection of fastidious bacteria in cardiac valves in cases of blood culture negative endocarditis. *J. Clin. Pathol.* **2001**, *54*, 238–240. [CrossRef] [PubMed]
25. Trinh, T.N.D.; Lee, N.Y. A foldable isothermal amplification microdevice for fuchsin-based colorimetric detection of multiple foodborne pathogens. *Lab Chip* **2019**, *19*, 1397–1405. [CrossRef]
26. Trinh, K.T.L.; Trinh, T.N.D.; Lee, N.Y. Fully integrated and slidable paper-embedded plastic microdevice for point-of-care testing of multiple foodborne pathogens. *Biosens. Bioelectron.* **2019**, *135*, 120–128. [CrossRef] [PubMed]
27. Mello, M.L.S.; de Campos Vidal, B. The Feulgen reaction: A brief review and new perspectives. *Acta Histochem.* **2017**, *119*, 603–609. [CrossRef]
28. Thai, D.A.; Lee, N.Y. A point-of-care platform for hair loss-related single nucleotide polymorphism genotyping. *Anal. Chim. Acta* **2023**, *1283*, 341973. [CrossRef] [PubMed]
29. Kampmann, M.L.; Børsting, C.; Morling, N. Decrease DNA contamination in the laboratories. *Forensic Sci. Int. Genet.* **2017**, *6*, e577–e578. [CrossRef]