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Manually operated microtube automatic capper/decapper system for clinical laboratory and biological laboratory personnel

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Abstract

Polymerase chain reaction (PCR) is an effective method for diagnosing infectious diseases and has been the primary method throughout the novel coronavirus disease (COVID-19) pandemic. PCR tests (from specimen collection to result acquisition) involve sample pretreatment, nucleic acid extraction, and PCR procedure. Automating the pretreatment process is crucial to mitigate the risk of infection for workers and to reduce the likelihood of sample contamination-triggered misdiagnosis, particularly when handling centrifuge tubes, cryopreservation tubes, and microtubes. Robotic systems have been engineered to automate cell culture and PCR-based diagnosis, predominantly designed for use with screw-capped containers. However, this leaves a notable gap in automation solutions for microtubes equipped with press-type caps. To address this gap, we developed a versatile microtube capper/decapper system. On the other hand, many tasks of manual operation using microtubes, which are routinely conducted in clinical tests and biological experiments, are performed. Compared to screw-type caps for centrifuge and cryopreservation tubes, press-type caps for microtubes present a considerably higher risk of the worker's fingers contacting the inside of the cap and/or generating airborne droplets. Despite the risks of contamination and infection derived from the manual handling of microtube caps, which can compromise diagnosis/experiment accuracy and worker safety, devices for manually opening and closing microtube caps without direct contact remain lacking. Therefore, leveraging the technology from the developed versatile microtube capper/decapper system for laboratory automation, we created a manually operated microtube equipped with an automatic capper/decapper system tailored for personnel in clinical and biological laboratories.

In this study, we first examined the required specifications and prerequisites for a manual microtube capper/decapper and clarified the operating methods, operating procedures, operation environment, device size, accompanying functions, etc. Based on the required specifications and preconditions, we proceeded with the mechanical and control design of the conceptual model, manufactured a prototype, and confirmed its basic functions and performance. The compliant to the required specifications and preconditions and the usefulness of the proposed manual microtube capper/decapper were validated through various experiments and demonstrations. Using the proposed microtube capper/decapper, even small-scale operations, which are challenging to streamline, can be performed nearly as efficiently as full manual operations. Although operation time was not reduced, the ability to open and close microtubes without manual contact is crucial for improving diagnostic and experimental accuracy and for reducing the burden on and enhancing the safety of laboratory personnel. Because microtubes are used in various clinical tests

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and biological experiments, we believe that the proposed system can markedly reduce the workload for personnel across numerous clinical and biological laboratories.

Keywords Laboratory automation, Mechanism design, Microtube, Clinical examination, Biological experiment, Manual operation

Introduction

Polymerase chain reaction (PCR) is an effective method for diagnosing infectious diseases and has been the primary method throughout the novel coronavirus disease (COVID-19) pandemic [1]. To accommodate newly emerging viruses and the mutants in the future, it is crucial to establish an inspection system that offers flexibility and continuity in both inspection operations and information processing. PCR tests (from specimen collection to result acquisition) involve a sample pretreatment, nucleic acid extraction, and PCR procedure. Automating the pretreatment process is significant due to the risks of infection for workers and potential misdiagnosis caused by sample contamination, particularly when handling centrifuge tubes, cryopreservation tubes, and microtubes.

Robotic systems for automating cell culture [2–4] and PCR-based diagnosis [5–8] have been developed, but these primarily cater to containers with screw caps, leaving a gap for systems compatible with microtubes with press-type caps. According to our knowledge, there are limited examples of automated systems incorporating microtubes with press-type caps [9–11]. Although capper/decapper systems for press-type caps exist [12–14], there is a notable lack of compact microtube cappers/decappers that accommodate a wide array of microtubes. Addressing this gap, we developed a versatile microtube capper/decapper system [15].

Moreover, in clinical tests and biological experiments, numerous tasks like centrifugation, vortexing, spinning down, and pipetting are conducted manually. Operations such as centrifugation, vortexing, and spinning down require closed microtube caps, while pipetting requires open caps. Compared to opening and closing screw-type caps for centrifuge and cryopreservation tubes, press-type caps for microtubes pose a considerably higher risk of the worker's fingers coming into contact with the inside of the cap and/or generating airborne droplets. Therefore, manual handling of microtube caps poses a contamination and infection risk, compromising both test/experiment accuracy and worker safety. Despite the existence of compact, manually operated devices for pipetting [16, 17], vortex mixing [18, 19], spinning down [20, 21] and capping/decapping of screw caps [22] that can be utilized within a biological safety cabinet, a device for manually opening and closing microtube caps without direct contact is absent. This underscores the need for a microtube capper/decapper capable of safely handling caps without direct contact.

In this paper, we first examine the required specifications and prerequisites for a manual microtube capper/decapper. We then proceed to design the mechanism and control of a conceptual model, followed by prototyping this model to confirm its basic functions and performance. The utility of the proposed manual microtube capper/decapper is demonstrated through various tests and demonstrations.

Methods

This section delineates the essential specifications and prerequisites for the manual microtube capper/decapper, followed by the mechanism and control design of a conceptual model, taking into account the needs of workers and researchers in biological experiments and clinical tests.

Conceptual design/basic design process

Required specifications and preconditions of conceptual design

To facilitate the conceptual design of the manual microtube capper/decapper, we established the following specifications and preconditions, reflecting the requirements of biological experiment and clinical test personnel:

1. Operators must manually insert and remove microtubes from the capper/decapper.
2. The device should accommodate the opening and closing of caps on both 1.5 mL and 2 mL microtubes.
3. It should allow for the insertion of microtubes with the cap in both open and closed states, as well as the removal.
4. The size, weight, and power source of the device should be compatible with use within a biological safety cabinet, and the device should be easy to carry (easy to put in and take out of the cabinet)
5. The operating procedures must be straightforward and simple. It must have a function to emergency stop the device when operators feel danger.
6. The device should permit visual inspection of the microtube's interior when the cap is in open and closed positions.

7. The device should be operable with a mobile battery.
8. It should be cleanable with alcohol or similar disinfectants.
9. The device with a cooling function for specimens and reagents within the microtube is preferable.
10. It should minimize the risk of contamination from specimen scattering, exposure to microorganisms, and aerosol generation during cap manipulation.

Taking these requirements and conditions into account, we designed a manual microtube capper/decapper.

Basic design of manual microtube capper/decapper body

This section focuses on the mechanism's structure. Figures 1 and 2 illustrate the manual microtube capper/decapper body and the microtube holder, respectively. The cap opening and closing mechanism is fundamentally similar to that of the automated microtube capper/decapper previously developed [15]. The output torque from the geared motor is transferred to a bevel gear, which drives the cap opening and closing arm. The maximum cap opening and closing force is approximately 80 N. However, we have made several key improvements to facilitate manual operation.

For manual operation, a switch box has been incorporated into the main body's front. The microtube, with an outer diameter of approximately 11 mm, is secured by a C-shaped holder near the top, which features a 9 mm gap at the front, and a stopper near the bottom, allowing for external observation of the microtube. This dual-point securing method ensures stability during cap manipulation. Additionally, this setup facilitates precise pipetting, as the operator can visually assess the spatial relationship between the pipette tip and the sample within the microtube. The stopper components are interchangeable, accommodating both 1.5 mL and 2 mL microtubes, as depicted in Fig. 2 (3) and (4). The C-shaped holder includes a support portion at the rear, leaving the area around the microtube, except for the upper portion, completely open. This design facilitates the installation of a cooling unit, which will be discussed later. Additionally, there is a guide section at the rear of the top surface of the C-shaped holder, enabling the microtube to be placed in the correct position and orientation by aligning it with this guide section (Fig. 2(5)). While the main body is not airtight, all electrical components, including the drive motor and sensors, are encased and can be cleaned with alcohol.

The cap is opened by elevating the lower surface of the cap's front protrusion using the opening arm. This action, synchronized with specific movements, facilitates a smooth cap opening process. As with the automated microtube capper/decapper [15], a push pin is positioned on the side of the C-shaped holder. However, the pressing mechanism and cap opening and closing arm have been made more compact, and additional space has been provided above the C-shaped holder to ensure that it does not interfere with the manual placement of the microtubes. The inner surface of the closure arm features two levels with a slope in between. As the closure arm rotates

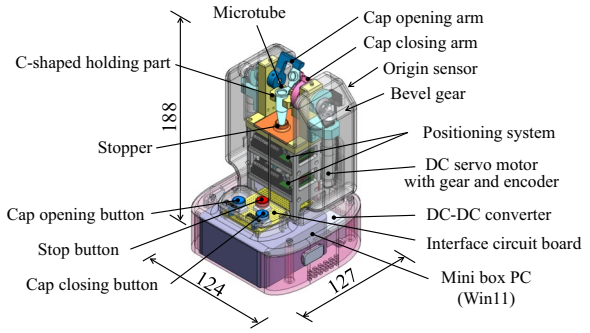

Fig. 1 Conceptual design of a manual microtube capper/decapper

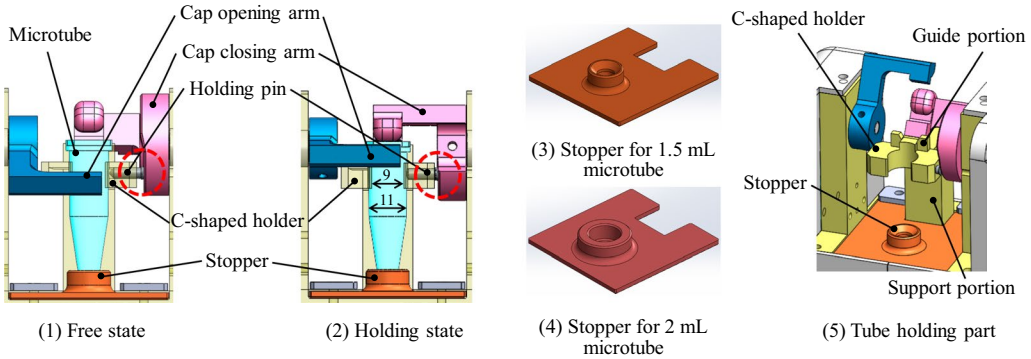

Fig. 2 Microtube holding mechanism and visibility

forward, the push pin transitions from the lower level, up the slope, to the higher level, exerting pressure on the microtube's upper side (Fig. 2 (2)). Moreover, during cap opening, the closing arm's tip gently presses the cap's top while the opening arm operates, preventing the cap from dislodging abruptly. This mechanism significantly reduces the risk of contamination through specimen or virus dispersal and aerosol generation during cap manipulation.

Figure 3 depicts the cooling function, which comprises a cooling box and a cooling material. The cooling box is designed to hold microtubes even though the front is open, allowing visibility into the microtube. Ice or frozen gel was used for the cooling material. Depending on the required cooling duration, a large or small cooling box may be selected. Although integrating a Peltier element-based cooling device was considered, to minimize the system's size and reduce power consumption, the cooling box and cooling material were synergized for the cooling function. This approach requires precooling the cooling material, but it allows for use only as needed, contributing to the system's compactness.

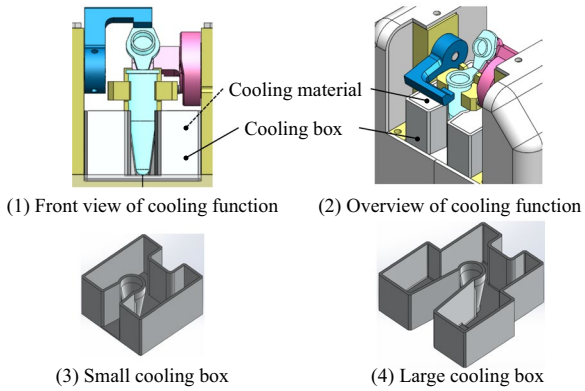

Fig. 3 Cooling function

Control system design

Figure 4 illustrates the control system. The microtube capper/decapper is governed by a mini box PC running Windows 11, paired with a motor position control system (Maxon EPOS2 24/2), both located at the device's base. The motor position control system receives operation signals for the opening, closing, and stop buttons through an interface circuit board. The device can be powered by a 100 V AC supply through an AC-DC converter ranging from 15 to 24 V DC, or by a mobile battery designed for laptop PCs. Within the device, a 12 V DC supply powers the mini box PC, the EPOS2, and the interface circuit board via a DC-DC converter.

Next, we will explain a series of operations. To commence operation, the power switch located at the rear of the microtube capper/decapper is activated, followed by the powering on of the mini box PC at the front. Subsequently, the capper/decapper's operating software initiates automatically, performs a homing sequence, and enters standby mode. An LED indicator near the operation buttons illuminates to signal readiness for operation.

Figure 5 illustrates the state transition diagram following the standby state, highlighting the relationship between switch operations and cap opening/closing actions. The operation follows a structured flow, with

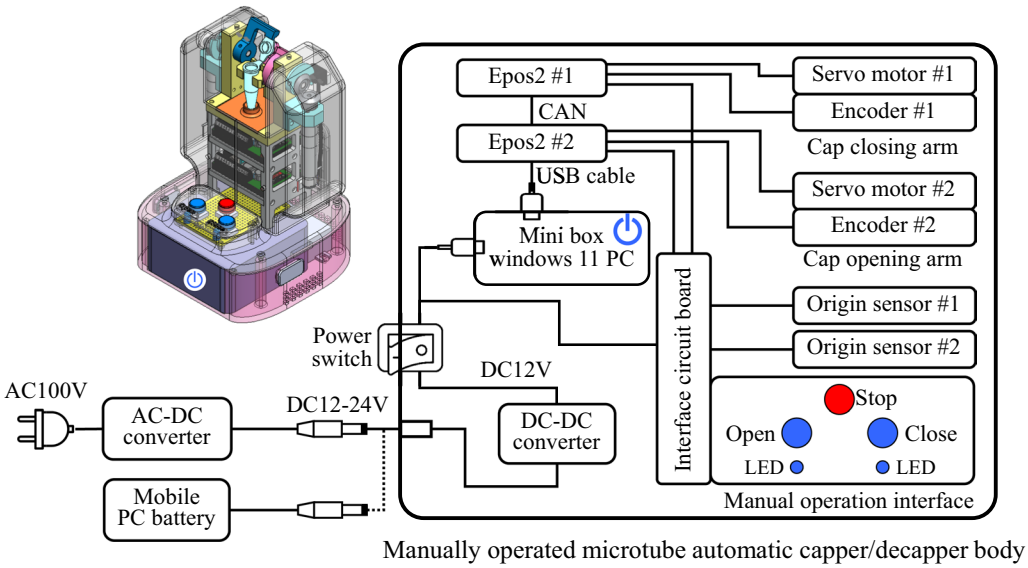

Fig. 4 Control system configuration

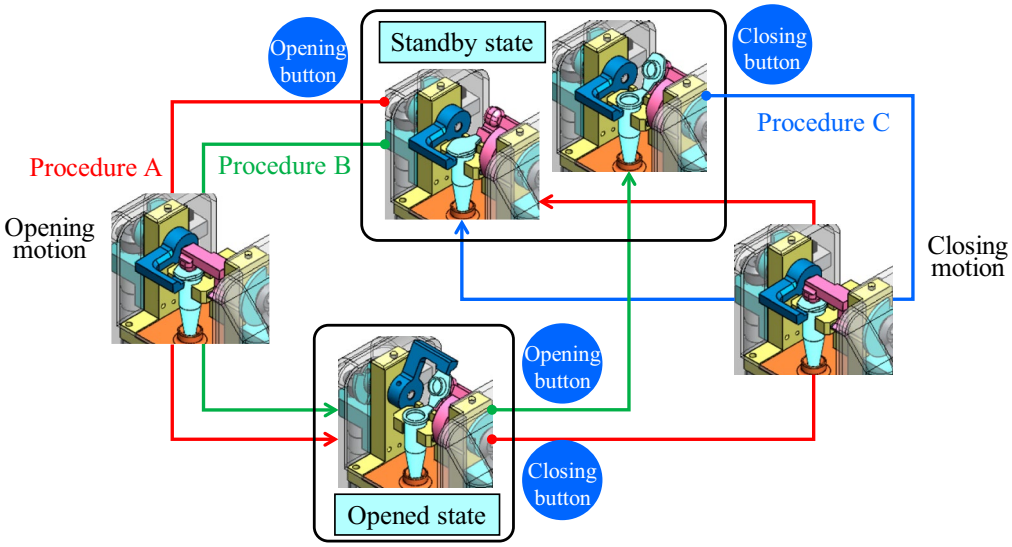

Fig. 5 State transition and operation procedure

three distinct procedures aligned with the established specifications:

1. Procedure A involves placing a microtube with a closed cap in the standby state. By pressing the opening button (blue button), the cap is opened, transitioning the tube to an opened state. Subsequent pipetting actions are followed by pressing the closing button (blue button) to seal the cap, reverting the system to the standby state for microtube retrieval. This sequence represents the standard operation.
2. Procedure B is initiated after opening the cap via Procedure A. Pressing the opening button again maintains the open cap state without transitioning to closure, effectively entering standby mode. This procedure is optimal for removing the microtube while keeping the cap open.
3. Procedure C caters to situations where a microtube with an open cap is set in the standby state. Pressing the closing button seals the cap, returning the system to standby, allowing for the microtube's removal. This procedure is dedicated to executing cap closure operations exclusively.

Regarding the described state transition, should you inadvertently activate an unintended operation, you can rectify this by subsequently pressing the appropriate button for your desired action, based on the current state of the system. This allows you to ultimately execute the intended operation. If it becomes necessary to halt the operation immediately, you can employ the stop button (red button) for this purpose. However, be advised that using the stop button will necessitate a system restart.

Figures 6, 7, and 8 show the motion patterns and respective speeds of the cap closing and opening arms for Procedures A, B, and C. The motion angles include machining errors and assembly errors of parts. The motion speeds (10 or 20°/s) are deliberately slow during the cap opening and closing operations to minimize the risk of positioning errors caused by heavy loads and to prevent contamination from droplet scattering, similar to the automated microtube capper/decapper system [15]. Conversely, the other operation speed (80°/s) is set higher than that of the automated system to reduce manual operation time. However, increasing the speeds of both arms any further, there is a risk that the emergency stop button will not be operated in time in the event of an operational error or trouble.

This conceptual and basic design framework enables the realization of a manually operated microtube automatic capper/decapper system that meets the specified requirements and conditions.

Results and discussion

This segment will detail the prototyping of a microtube capper derived from the aforementioned design, alongside evaluations of mechanical functionality and usability, ensuring compliance with the specified requirements and conditions.

Prototype model

Figure 9 presents a prototype of the proposed manually operated microtube automatic capper/decapper system,

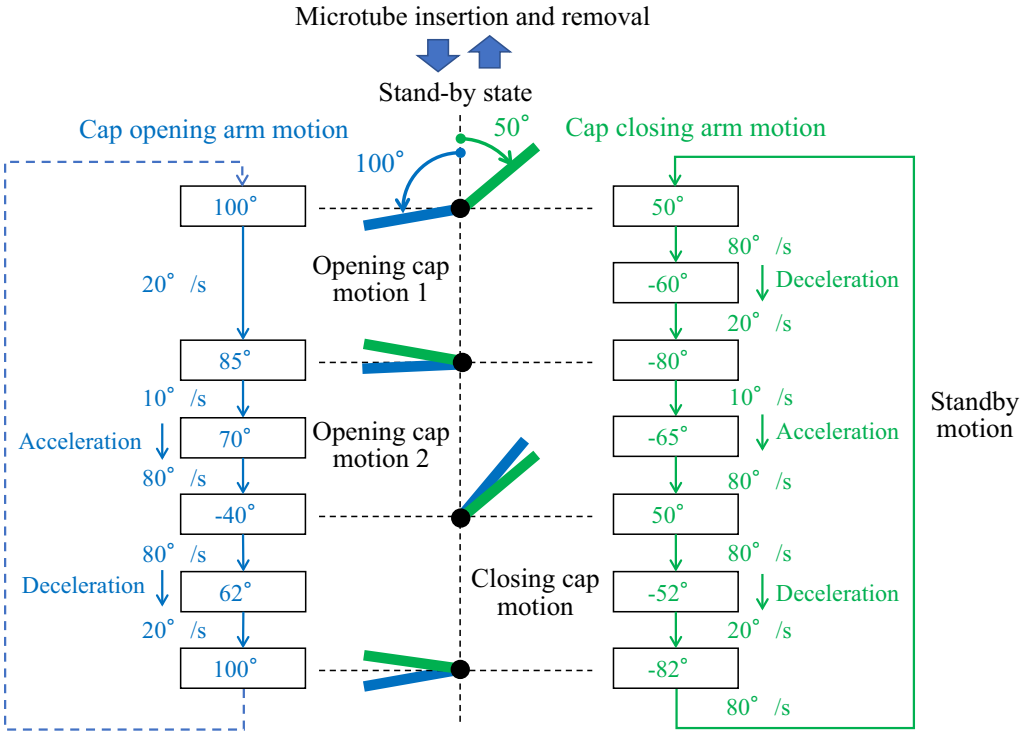

Fig. 6 Motion of cap opening and closing arms in Procedure A

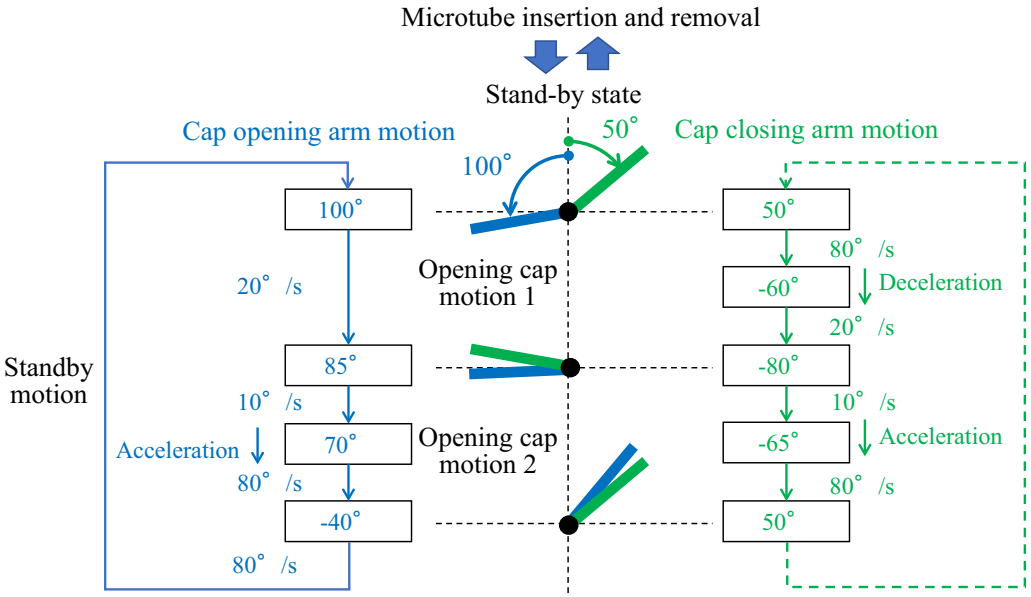

Fig. 7 Motion of cap opening and closing arms in Procedure B

conforming to the mechanical design outlined earlier. The front panel hosts the mini box PC switch, with the power supply connector and switch positioned at the rear. The stopper for a 2 mL microtube is placed on the back side. The prototype's total weight is approximately 1,330 g, underscoring its compact and lightweight nature for facile transportation within a biological safety cabinet. It facilitates direct visual inspection of the microtube's interior, whether the cap is open or closed. The exposed metal parts are primarily constructed from anodized

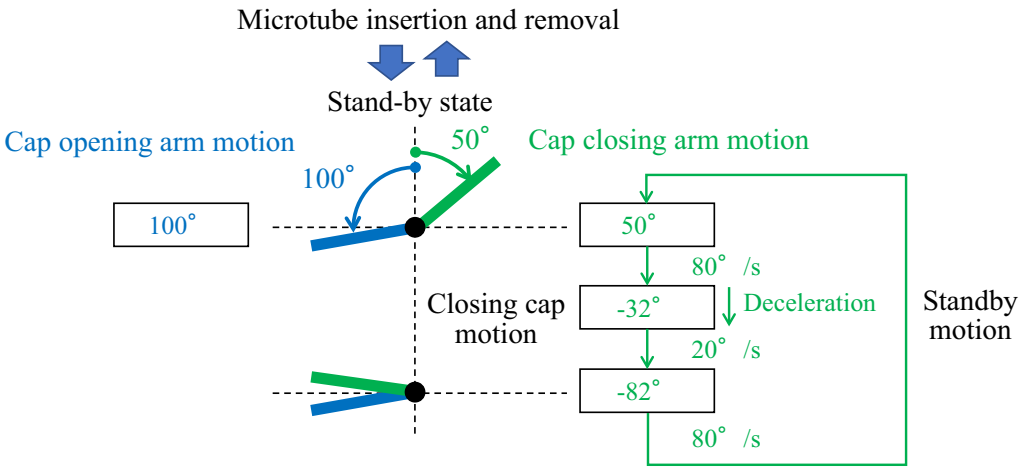

Fig. 8 Motion of cap opening and closing arms in Procedure C

Fig. 9 Overview of a prototype of the proposed manually operated microtube automatic capper/decapper system

aluminum alloy or stainless steel, and the covers are 3D-printed parts (printed by Stratasys F170, 333–60,300 ABS-M30 (Ivory)). Particular damage was not observed after wiping and spraying with disinfectant alcohol.

Evaluation of opening and closing cap function

The function of the opening and closing cap of the microtube was evaluated. The microtubes used this time were Thermo Fisher #3448 (1.5 ml) and Greiner 623,201 (2 ml). We verified that when the microtube capper/decapper was powered by a 15 to 24 V DC supply, connected through an AC–DC converter from a 100 V AC source, and linked to a mini box PC, the device's operating program for manual capping and decapping automatically initiated. It executed a return-to-origin operation and then entered a standby state. It took about 30 s from powering on the mini box PC to the standby state. Furthermore, we confirmed that a series of operations, such as Procedures A–C, can be performed by pressing the open and close buttons as appropriate after setting the microtube in the standby state. Figure 10 shows a series of opening and closing operations using the manual

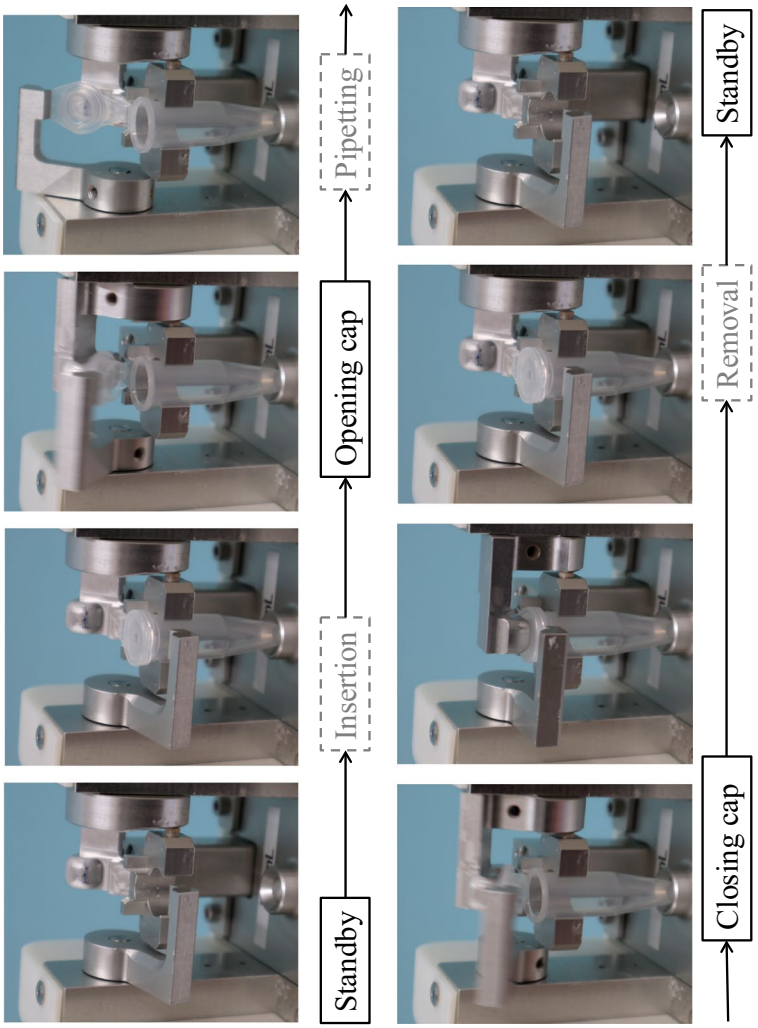

Fig. 10 Opening and closing cap motion (Procedure A)

microtube capper (Procedure A). We also verified that the opening and closing operations proceeded smoothly without issues, with the times recorded as 5.4 s for opening and 4.9 s for closing. However, if microtubes are not correctly oriented, aligned with the guide, or placed properly, the cap may fail to open or close. In such cases, the operation can be stopped immediately by pressing the stop button. The operation could be stopped immediately by pressing the stop button. Furthermore, it was driven in the same way by a mobile battery designed for notebook PCs (SANWA SUPPLY INC., 700-BTL033BK, DC12 V, 16 V, 19 V (3.6A) 17400mAh, 62.64Wh, and 700-BTL049 DC12 V (3.6A) 17400mAh, 62.64Wh).

Evaluation of the operation time of pipetting tasks

The working times were compared between the full manual operation and the manual operation using the microtube capper/decapper. The comparison tasks were determined with reference to the procedures performed in the sample pretreatment process of the PCR-based diagnosis used at the Kawasaki City Institute for Public Health. As shown in Fig. 11, the procedure involves dispensing 150 µL from a cryopreservation tube containing 1000 µL of specimen with phosphate-buffered saline (PBS) into a microtube containing 600 µL of Lysis Buffer RAV1. Each lot consists of 12 samples. Transferring samples from cryotubes to microtubes using a pipette is not only a preparatory step for PCR-based diagnostics but also one of the most common tasks. Figure 12 shows the pipetting task sequences of full manual operation and manual operation using the microtube capper/decapper. In the comparison task, tap water was used instead of specimens and reagents. Consequently, the evaluation pipetting task involves dispensing 150 µL from a cryopreservation tube (SARSTEDT 72. 694. 100. 02, 2 mL) containing 1000 µL of tap water into a microtube: (Thermo Fisher #3448, (1.5 ml) containing 600 µL of tap water. Similarly, each lot consists of 12 samples. Figure 13 shows the arrangement of cryopreservation tubes and microtubes and opening/closing cap and aspiration/dispensing position. The tubes before aspiration/dispensing are arranged on the right side, and the tubes after aspiration/dispensing are arranged on the left side.

The experimental setup is shown in Fig. 14. Before starting the experiment, a subject (right-handed, nonprofessional), one of the authors, was required to familiarize himself with the pipetting task. The subject ran four trials (12 pieces × 4 lots) for each condition, each of which was video-recorded. Each operation time shown in Fig. 12. (handling and opening cap time, pipetting time and closing cap and the handling time) was retrieved based on the video.

Figure 15 shows the experimental results of handling and opening cap time, pipetting time and closing cap and handling time by box plots, and the average operation time of one piece by bar graph. Due to some operational sequence errors in the first trial, the box plot data excluded the operation times affected by these errors. However, it was included in the average working time per piece. The first trial had a large variation, but the second to fourth trials had a smaller variation by learning curve. The time required to open and close the cap, including tube handling, was approximately 1–2 s longer when using the microtube capper/decapper relative to the full manual operation. No significant difference existed in dispensing time. In pipetting tasks, after opening and closing the cap of a microtube, additional steps often include opening and closing caps of other containers, attaching and detaching tips, and aspiration and dispensing. By optimizing the task procedure, these activities can be performed concurrently. Therefore, the approximately 5 s required to open and close the microtube cap is expected to have minimal impact on the overall process time. Generally, automated equipment for small lot work is inefficient, and manual methods are typically faster, leading to limited on-site acceptance. In contrast,

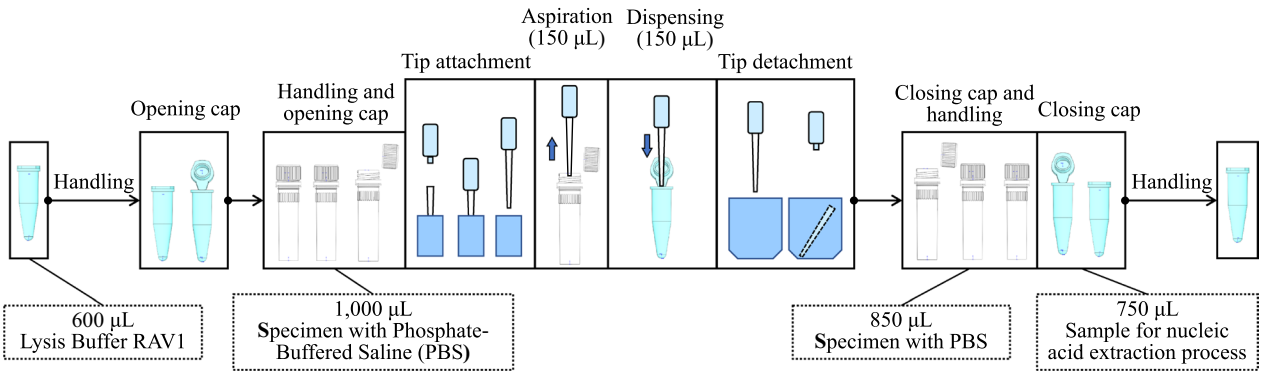

Fig. 11 Pipetting task sequences of the pretreatment process of the PCR-based diagnosis used in Kawasaki City Institute for Public Health

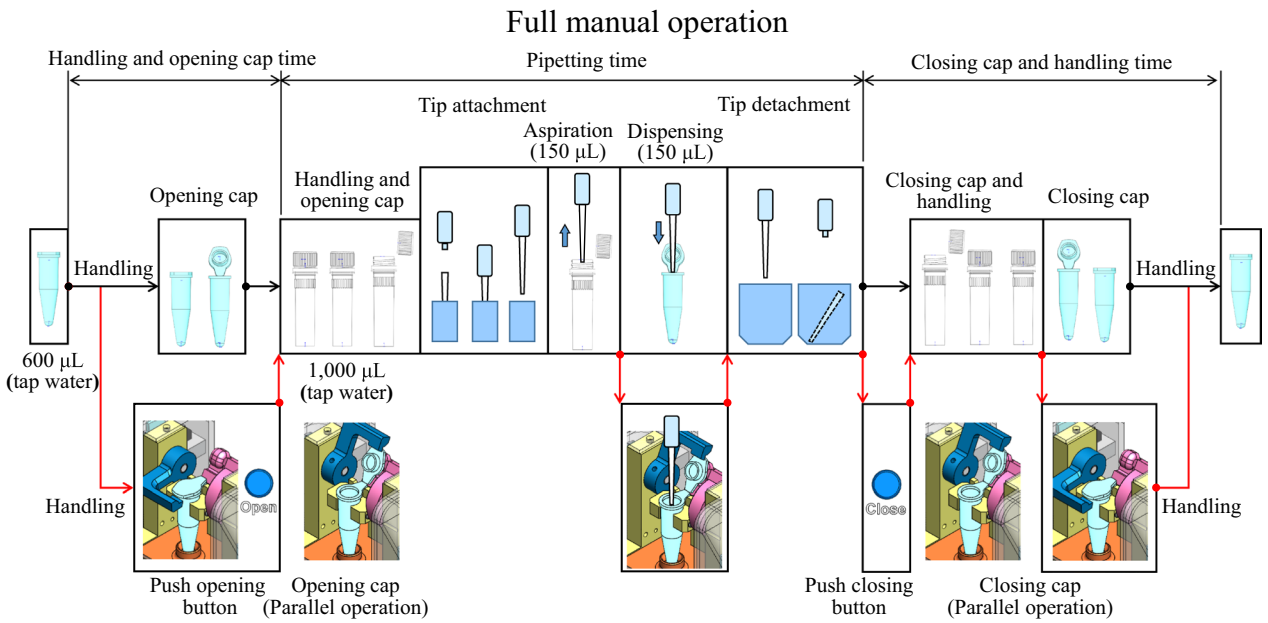

Fig. 12 Pipetting task sequences of full manual operation and manual operation using the microtube capper/decapper

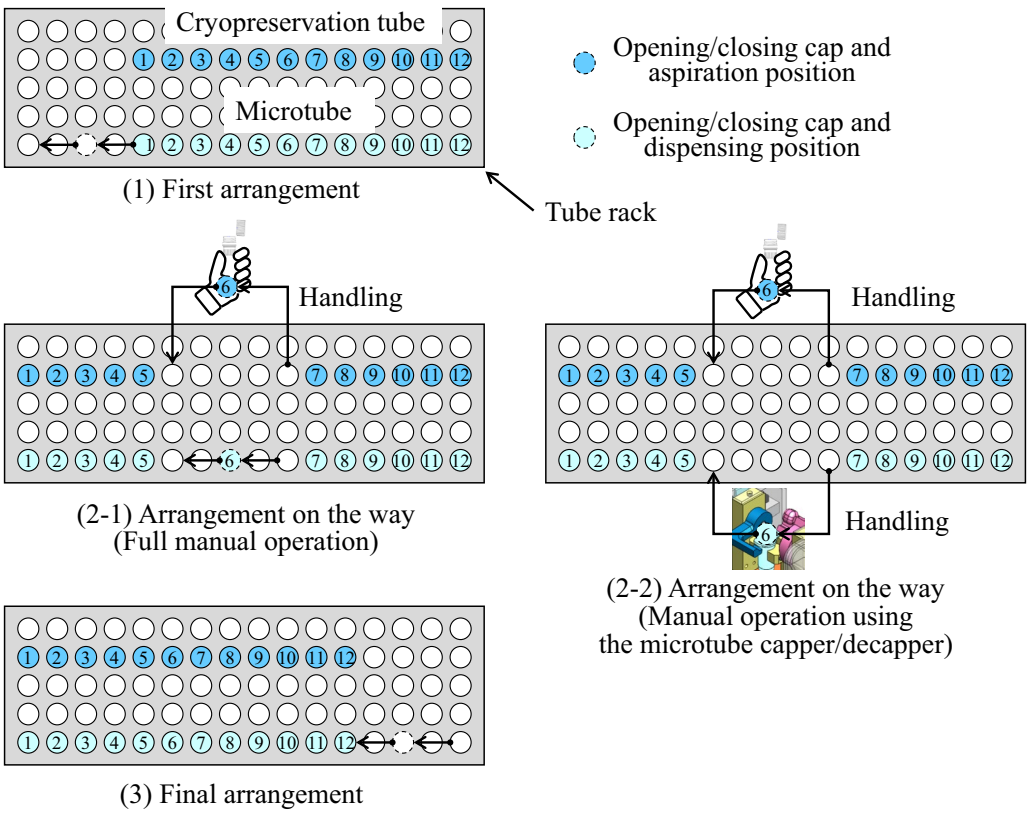

Fig. 13 Arrangement of cryopreservation tubes and microtubes and opening/closing cap and aspiration/dispensing position

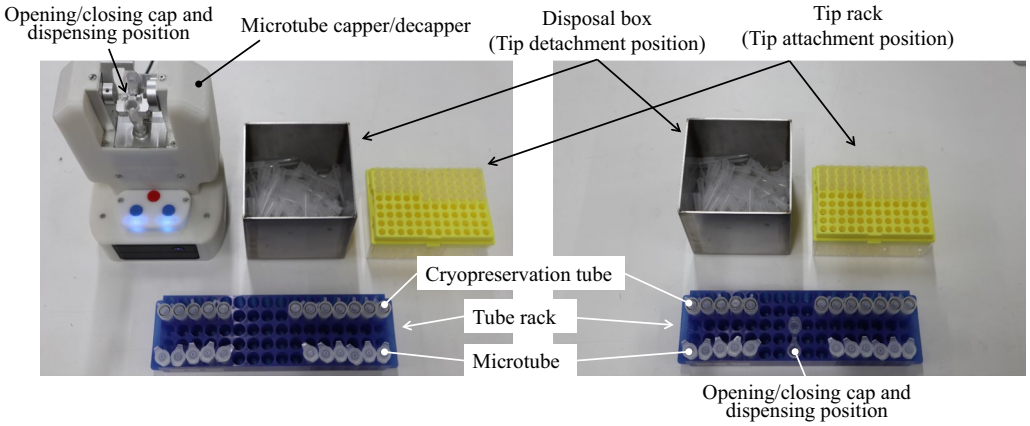

Fig. 14 Experimental setup for evaluation of operating time of pipetting tasks

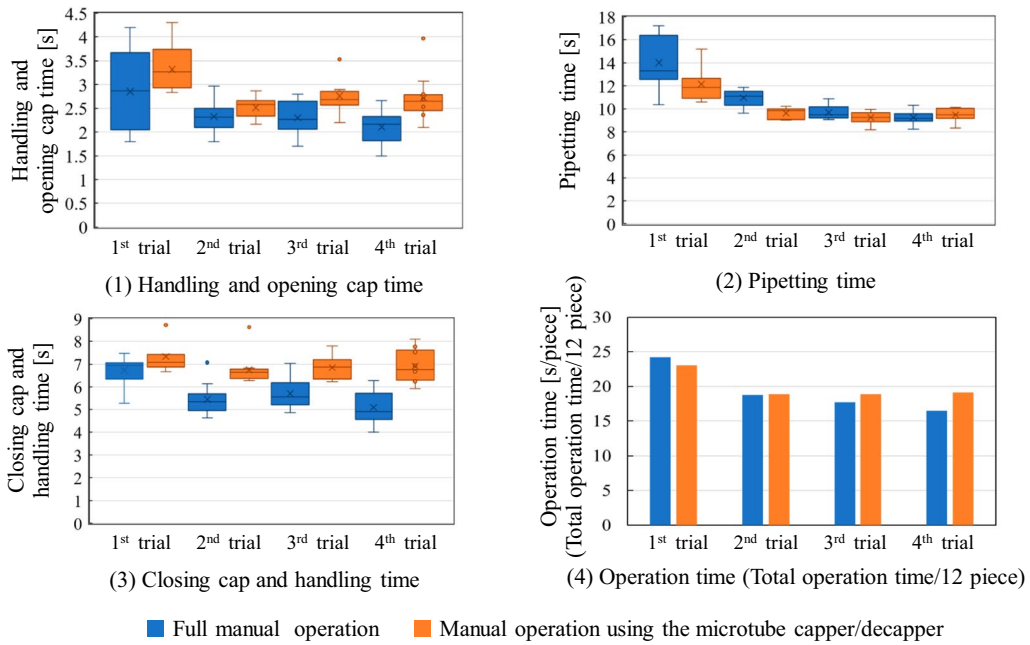

Fig. 15 Experimental results of handling and opening cap time, pipetting time, closing cap and handling time and average operation time of one piece

the proposed microtube capper/decapper operates in a comparable time frame, even for tasks involving up to 12 pieces per lot. This is a noteworthy point. One approach to considerably increase throughput is to use multiple microtube capper/decappers or to develop a device capable of opening and closing several microtubes simultaneously. However, because pipetting tasks may involve various types of tubes, it is essential to consider the entire process to enhance overall efficiency. This evaluation of the cap opening and closing function did not lead to a reduction in operation time. However, the ability to open and close microtubes without manual contact is crucial for enhancing diagnostic and experimental accuracy and for reducing the burden on and ensuring the safety of laboratory personnel.

Evaluation of the cooling function

The cooling function using the cooling boxes was evaluated. The cooling box was prototyped by a 3D printer (printed by Formlabs Form 3, Resin Rigid 4000 V1).

The capacities of the small and large cooling boxes were 32.3 mL and 55.5 mL, respectively. Figure 16 shows the experimental conditions for the cooling function. The experiments were conducted under four conditions: (1) No-cooling box sample, (2) Small cooling box sample (Tap water ice), (3) Small cooling box sample (frozen gel), and (4) Large cooling box sample (Tap water ice). Furthermore, under all experimental conditions, room temperature and left indoor samples were simultaneously measured. The temperature was measured by K-Thermocouple (HIOKI 9810) in the microtube bottom and middle position and recorded by a data logger (HIOKI LR8431). An hour of data was acquired. Tap water ice or frozen gel was used as the cooling material. After putting tap water or gel (Contents of Snow Pack R-20 by MIE Chemical Industry) in the cooling box and cooling it in a freezer (−17℃) to freeze, experiments of the cooling functions were conducted. A 1,000 µL of tap water in the microtube: Thermo Fisher #3448 (1.5 ml) was cooled to approximately 0 °C–4 °C with crushed ice. Figure 17 shows the experimental setup for the cooling functions.

Figure 18 shows the experimental results of the cooling functions as the average value of time series data acquired three times under the same conditions. The room temperatures were 22 °C − 23 °C in all cases, and the experiments were conducted in almost the same temperature environment. In the case of the no-cooling function, the temperature rises after the measurement starts,

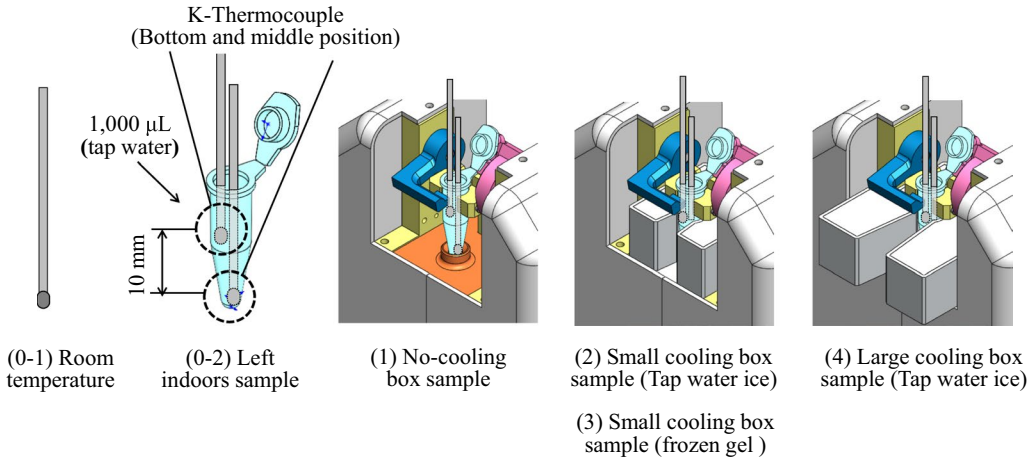

Fig. 16 Experimental conditions for cooling function

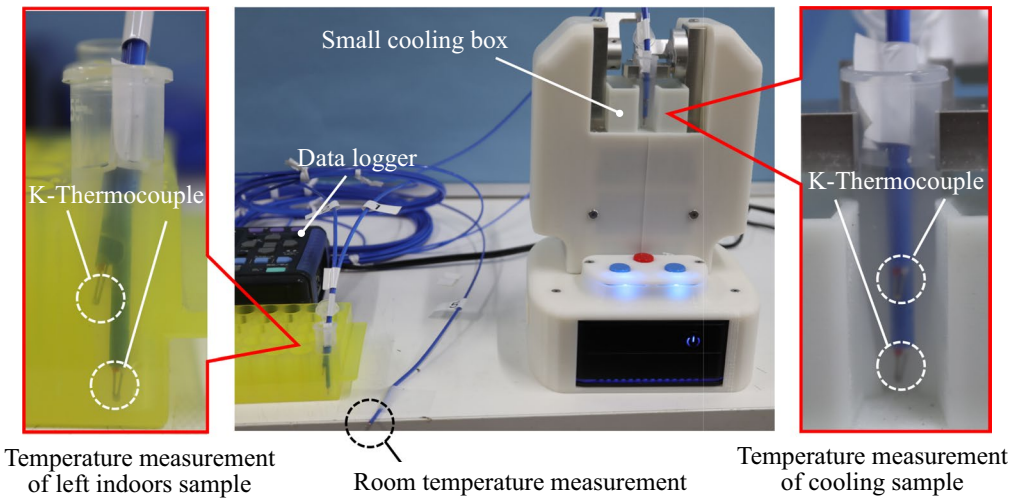

Fig. 17 Experimental setup for cooling function

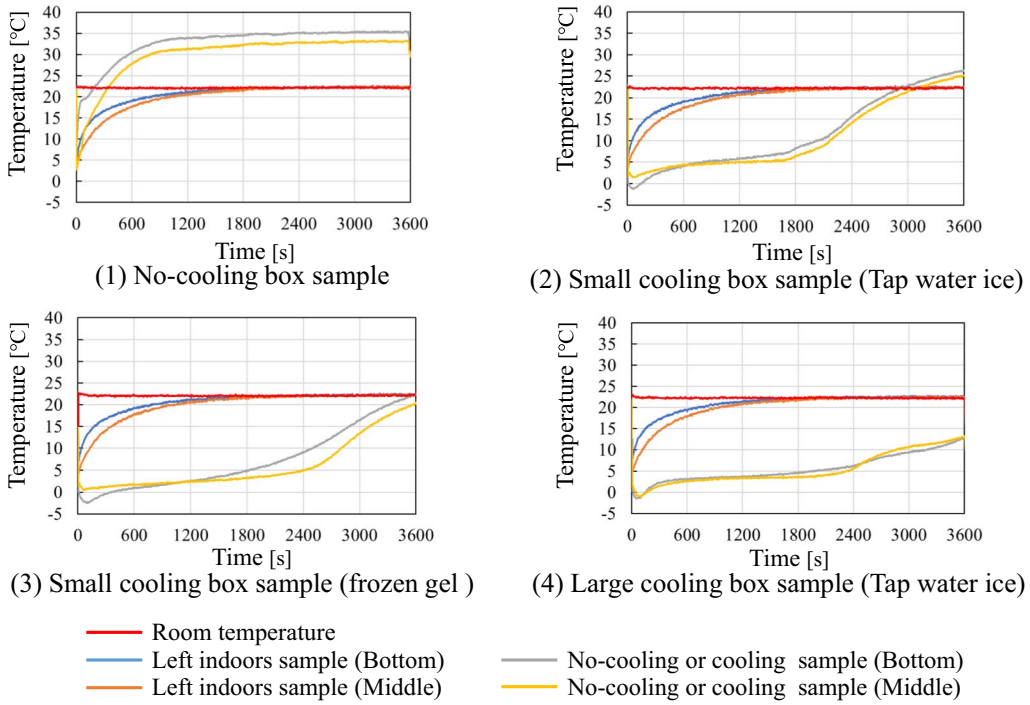

Fig. 18 Experimental results of the cooling functions

rising to about 35℃ in 20–30 min, and then remaining almost constant. The heat sources are thought to be the mini box PC and motor position control system (Maxon EPOS2 24/2). In contrast, using the cooling box caused the temperature of the tap water in the microtube to be maintained at about 5℃ or less for about 20–40 min. Although no noticeable difference was observed between the tap water ice and the frozen gel, the cooling function was maintained for a long time, depending on the difference in capacity between large and small boxes. While precise temperature control over long periods proved challenging with a cooling box–based cooling function, it effectively sustained the coolness of the microtube contents for short durations. The cooling performance is expected to improve through methods such as inserting a heat-insulating material between heat sources (the mini box PC and the Maxon EPOS2 24/2) and placing cooling material under the microtube.

Discussion on required specifications and preconditions

The considerations for the abovementioned requirement specifications and preconditions are summarized below.

1. Operators can manually insert and remove microtubes from the capper/decapper.
2. The device accommodates the opening and closing of caps on both 1.5 mL and 2 mL microtubes.
3. Using procedures A, B, and C, microtubes can be inserted and removed with their caps in both the open and closed states.
4. The size, weight, and power source of the device are compatible for use within a biological safety cabinet, and the device is easy to carry (easy to put in and take out of the cabinet)
5. The operating procedures are straightforward and simple, with only pushing buttons.
6. This device allows visual observation of the microtube's interior when the cap is in open and closed positions.
7. The device is operable with a mobile battery.
8. It is cleanable with alcohol or similar disinfectants.
9. The device has a cooling function for specimens and reagents within the microtube.
10. Quantitative evaluations of the risk of contamination from specimen scattering, virus exposure, and aerosol generation during cap manipulation are currently being conducted and will be reported separately.

Conclusion

Manual handling of microtube caps poses a contamination and infection risk, compromising both diagnosis/experiment accuracy and worker safety. However, a device for manually opening and closing microtube

caps without direct contact is absent. Therefore, leveraging the technology of our initially developed versatile microtube capper/decapper system for laboratory automation, we have developed a manually operated microtube equipped with an automatic capper/decapper system for clinical and biological laboratory personnel. The suit to the required specifications and preconditions and the usefulness of the proposed manual microtube capper/decapper were validated through various experiments and demonstrations. It is noteworthy that the proposed microtube capper/decapper operates at nearly the same time as manual handling, even for small batches. Although the operation time was not reduced, the ability to open and close microtubes without manual contact is crucial for enhancing diagnostic and experimental accuracy and for reducing the burden on and ensuring the safety of laboratory personnel.

In the future, we plan to perform quantitative evaluations of contamination risk using the proposed microtube capper/decapper system. Furthermore, we plan to proceed with the social implementation of the proposed system. Given the extensive use of microtubes in diverse clinical and biological experiments, we believe that the proposed system can markedly reduce the workload of personnel across numerous clinical and biological laboratories.

Abbreviations

PCR Polymerase chain reaction
LED Light-emitting diode

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40648-024-00281-3.

Additional file 1.

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Author contributions

M.J. spearheaded the conceptual planning and design, the mechanical design, and the verification experiments for evaluations, in addition to drafting the initial manuscript. R.N. was instrumental in developing the control system, along with the design and implementation of the control software. Y.S., R.Y., T.K., and J.Y. were pivotal in defining the specifications and prerequisites for the conceptual design and carried out usability evaluations of the prototype for the proposed manually operated microtube automatic capper/decapper system. All authors have reviewed and endorsed the final manuscript.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare no competing interests.

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