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3D Printer-Based PCR Reagent Dispenser with Syringe Pump and Three-Way Valve for Rapid Nucleic Acid Diagnostics

Chi-Uk Han[1], Jongwon Kim[2], Mun-Ho Ryu[3,*]

[1]Department of Healthcare Engineering, Jeonbuk National University, Jeonju, Republic of Korea

[2]Biomedux Co., Ltd., Gyeonggi, Republic of Korea

[3]Division of Biomedical Engineering; Research Center of Healthcare & Welfare Instrument for the Aged, Jeonbuk National University, Jeonju, Republic of Korea

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Abstract

This study presents a cost-effective automation solution for preparing polymerase chain reaction (PCR) reagent cartridges used in automated nucleic acid analyzers. Manual preparation is labor-intensive and error-prone, often causing inaccurate volumes and reagent mismatches. To address this, a dispensing system based on open-source 3D printer technology is developed. It incorporates a motion platform and a syringe-based pump, and precisely dispenses reagents into cartridge chambers designed for magnetic DNA extraction and real-time PCR. The system is evaluated for manual inefficiency and error. Dispensing accuracy, assessed gravimetrically using 500 μL of distilled water, shows a relative accuracy of 0.30% and a coefficient of variation (CV) of 2.64%, both within ISO 8655 limits. In terms of efficiency, the system fills a single cartridge chamber in 13.57 seconds, much faster than the approximately 3 minutes required for manual reagent injection. These results highlight the system's potential to improve throughput and precision in cartridge preparation.

Keywords: liquid handling, reagent dispenser, nucleic acid diagnosis, 3D printer

1. Introduction

Accurate and timely diagnosis of infectious diseases is essential for effective clinical and public health responses. While methods such as virus culture, serological testing, and nucleic acid detection are used, real-time quantitative polymerase chain reaction (qPCR) is recognized as the most accurate and sensitive method [1-6]. It enables real-time quantification of DNA or RNA during amplification and has become the gold standard for molecular diagnostics, including COVID-19 testing [6]. To support rapid and accessible diagnostics, Kil et al. [7] developed a low-cost, portable nucleic acid analyzer that automates nucleic acid extraction, qPCR, and result interpretation using a microfluidic cartridge. This cartridge includes a central syringe pump and 13 surrounding reagent chambers connected by rotary valves. Once the sample is loaded, the device carries out the entire testing process autonomously.

However, the manual pre-filling of reagents into cartridge chambers remains a bottleneck, limiting consistency, throughput, and scalability. Automating this liquid handling step is essential for transitioning the system into practical, high-volume applications. In recent years, advances in robotics [8] and automation technologies have led to the development of diverse liquid handling systems aimed at improving precision, reproducibility, and throughput in laboratory workflows. These

* Corresponding author. E-mail address: mhryu@jbnu.ac.kr

systems include both high-end commercial robotic platforms and cost-effective devices constructed using open-source components or adapted from 3D printer hardware. Such platforms have been widely applied across fields, including clinical diagnostics [9-13], life sciences [14-17], chemistry [18], bioanalysis [19], bioprinting [20], and genetics [21-22].

For example, Kopyl et al. [18] developed a pipetting system by modifying a desktop 3D printer, while Gome et al. [20] introduced a modular open-source liquid handling platform for bioprinting applications. Commercial open-source platforms such as Opentrons [22] have further demonstrated the potential of accessible lab automation, while other studies have shown that repurposing low-cost 3D printers can serve as a practical foundation for custom dispensing systems [23]. These studies show that robotic liquid handling—whether using high-end or low-cost hardware—can effectively address the limitations of manual processes, offering enhanced reproducibility and scalability for complex fluidic operations.

Despite these advances, most systems are not tailored to the specific design of microfluidic cartridges with rotary valves, which require coordinated control of multi-chamber reagent delivery. To address this gap, a 3D printer-based reagent dispensing system has been developed. It is specifically designed for polymerase chain reaction (PCR)-compatible microfluidic cartridges. The system includes a three-axis motion stage running open-source firmware, a syringe pump with a 3-way distribution valve, and a PC-based control interface. Performance evaluation indicates that this approach provides a cost-effective and accurate robotic solution for automating reagent preparation in nucleic acid diagnostic workflows.

2. Methodology

This section describes the methodology for developing an automated reagent dispensing system for PCR reagent cartridges. The system integrates a 3-axis motion table for accurate nozzle positioning at cartridge inlets, and a syringe pump for precise liquid aspiration and dispensing from reservoirs. These mechanical components are controlled through a PC-based user interface (UI) application. The design aims to overcome the limitations of manual cartridge preparation, ensuring high accuracy and improved efficiency in liquid handling.

2.1. Design of mechanical systems

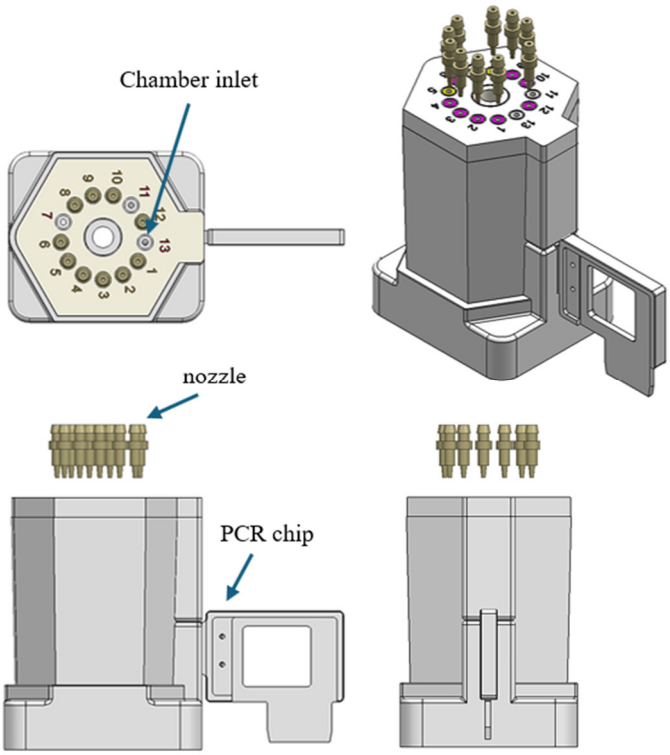

Fig. 1 Microfluidic cartridge with zozzles

As briefly mentioned in the introduction, the microfluidic cartridge used in this study contains 13 independent chambers. According to the current diagnostic protocol, eight different reagents, each with a volume ranging from 30 µL to 410 µL, must be preloaded into the chambers. Each chamber inlet has a diameter of approximately 1 mm; therefore, a custom nozzle was designed to connect a 2 mm diameter tube from the syringe pump outlet (Fig. 1). A nozzle unit was constructed, comprising 10 nozzles arranged at approximately 5 mm intervals to align with the chamber positions on the cartridge. This nozzle unit replaces the hot end of the 3D printer and is positioned at the dispensing location by the 3D motion table.

To enhance reagent dispensing throughput, a flat tray capable of holding up to 20 cartridges was fabricated (Fig. 2). The tray measures 300 × 240 × 18 mm and has pockets arranged in a 5 × 4 grid, each designed to securely hold a cartridge. Loading 20 cartridges onto the tray takes approximately 1 minute, and tray replacement requires about 1 minute.

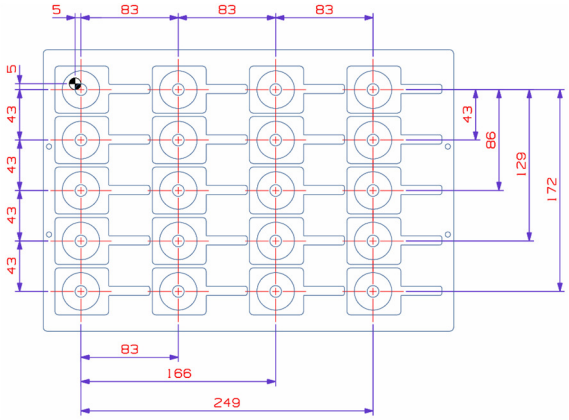

Fig. 2 The flat tray capable of holding 20 cartridges

A three-dimensional motion table is required to adjust the relative position between the flat tray and the nozzle unit. In this study, a low-cost computer numerical control (CNC) router frame (CNC 3018, HAEYON, China) was repurposed as a 3-axis motion table (Fig. 3). The router's working area (300 × 180 × 40 mm) accommodates the 249 × 172 mm movement range of the tray.

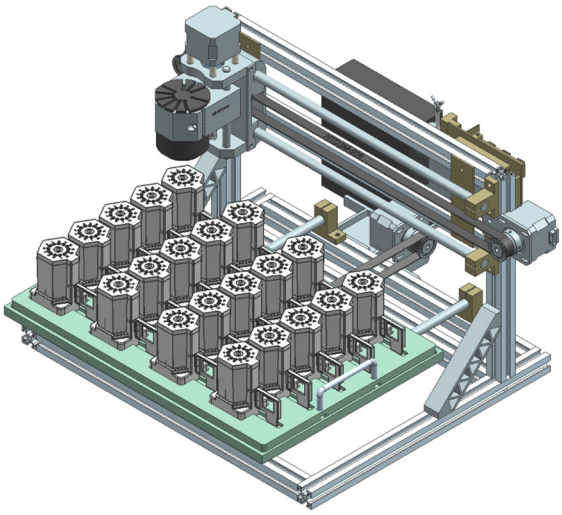

Fig. 3 The 3-axis motion table with a nozzle unit and flat tray

To improve the movement speed, the original screw-driven X and Y axes were replaced with a timing belt system. The original GRBL (an open-source G-code interpreter for CNC machines)-based controller was replaced with the open-source 3D printer control board RepRap Arduino Mega Pololu Shield (RAMPS) 1.4 and Marlin firmware (bugfix-2.1x), which was minimally modified for this application while retaining core functionalities. RAMPS 1.4 is a widely used open-source control board originally designed for desktop 3D printers. It is mounted on an Arduino Mega microcontroller and allows for easy integration of stepper drivers and other peripherals such as heaters, fans, and endstops.

Marlin is an open-source firmware developed for 3D printing applications, offering precise motion control, temperature management, and G-code interpretation. G-code is a standardized set of commands used to control automated machine tools, where each instruction specifies actions such as moving to a particular position, setting speeds, or turning components on or off. In this study, Marlin's core motion control functionality was leveraged to operate the motorized stages through standard G-code commands.

The Marlin firmware was slightly modified to support precise reagent dispensing while maintaining standard 3D printer functions. Key adjustments included setting the steps per millimeter for each axis based on hardware specifications. The X and Y axes achieved a resolution of 20.62 µm/step, taking into account the pulley teeth, belt pitch, and stepper motor settings. The Z-axis was configured for finer control, resolving 1.25 µm/step through a combination of lead screw pitch and microstepping. Key mechanical configuration parameters are summarized in Table 1.

Table 1 Mechanical configuration parameters

Axis	Stepper motor (Steps/Rev)	Microstepping	Transmission type	Pulley pitch/teeth	Steps per mm	Resolution (µm/step)
X, Y	200	1/16	Timing Belt	3 mm pitch, 22 teeth	48.48	20.62
Z	200	1/4	Lead Screw	1 mm pitch	800	1.25

2.2. *Syringe pump*

For liquid reagent handling, a pump capable of both aspirating and dispensing liquids is required. A syringe pump without a valve, similar to a pipette, must move to the reagent reservoir to aspirate the reagent and then to the dispensing position to release it, repeating this process for each operation. However, using a syringe equipped with a valve offers advantages, as the valve selection allows both aspiration and dispensing, eliminating the need to move to the reservoir for aspiration.

In this study, a commercially available syringe pump with a valve (Cadent™ 3, IMI Norgren, United Kingdom) was employed. This syringe pump consists of a syringe drive (PN 100941), a syringe (PN 23168), and a 3-way distribution valve (PN 25394). The syringe drive provides linear motion with a 30 mm stroke via a stepper motor (200 steps per revolution), a stepper motor driver (2 half-steps per step), and a 2 mm lead screw. A full 30 mm stroke of the syringe drive corresponds to 6,000 half-step commands. The syringe itself has a capacity of 1,000 µL with a 30 mm stroke. The syringe pump has a built-in control unit and provides serial communication functionality, which was utilized to control the pump in this study. It offers excellent accuracy (0.2% for a full stroke and 0.3% for a 1/10th stroke) and precision, expressed as the coefficient of variation (CV), with 0.05% for a full stroke and 0.5% for a 1/10th stroke. Additionally, the 1,000 µL capacity syringe is well-suited to the reagent volumes used in the microfluidic cartridges.

2.3. *Overall system configuration*

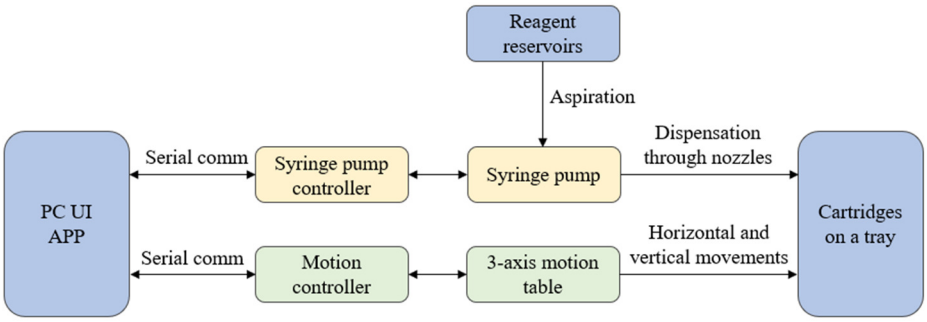

Fig. 4 The overall system configuration

The automated reagent dispensing system proposed in this study consists of a 3-axis motion table, a syringe pump, and a PC UI application. The 3-axis motion table controls the horizontal positioning of the flat tray holding the cartridges, as well as the vertical movement of the nozzle unit connected to the syringe pump (Fig. 4). The syringe pump dispenses reagents through

the nozzle into the chambers of the cartridges. The PC UI application allows for manual operation of the 3-axis motion table and nozzle unit via serial communication, or it can automatically dispense reagents into the cartridges according to a protocol written in a text file. This setup provides both manual control for flexibility and automatic operation for consistency and efficiency, making it well-suited for precise and high-throughput reagent dispensing in microfluidic cartridge applications.

2.4. 3-axis motion table interface

To drive the 3-axis motion table through the PC UI application, a communication interface based on Marlin firmware was employed. Marlin processes G-code commands sent from the application and returns appropriate status messages, enabling synchronization between the motion controller and the host system.

In this study, a set of essential G-code instructions was utilized to control axis movements, perform homing operations, and verify motion completion. While Marlin typically responds with a simple acknowledgment for each command, additional messages such as periodic status updates and position reports are used during longer operations. A summarized list of the G-code commands used in this system and their corresponding response behaviors is shown in Table 2. The ok message confirms command acceptance. The Alive message indicates ongoing operations, and the Position message provides current coordinates. The PC UI application uses M400 to confirm that the movement is fully complete before proceeding.

Table 2 Summary of G-code commands and responses

Command	Description	Response behavior
G00 X Y Z	Linear axis movement	Immediate ok, periodic Alive, final Position
G28	Auto homing	Alive every 2s during homing, final Position, then ok
M400	Wait until all moves are finished	Blocks until all movements are complete, then ok

2.5. Syringe pump interface

The Cadent™ 3 syringe pump controller, similar to Marlin firmware, operates using line-based DT protocol commands terminated with a newline (\n). Each command starts with a forward slash (/), followed by a slave address (0x0–0xf), a command string, and ends with a newline character. This study analyzed the protocol by referencing the manufacturer's GUI and interface documentation.

The core functionalities of the syringe pump controller are summarized in Table 3. These include valve selection and precise syringe positioning for fluid handling. The 'o' command is used to rotate the valve, while the '?' command periodically checks the controller status and syringe position. The response status byte distinguishes between idle (`` ` ''), busy ('@'), and error states (numeric code). Syringe movement is controlled via commands such as 'P' (aspirate), 'D' (dispense), and 'A' (absolute positioning). These commands enable accurate and synchronized fluid manipulation, which is essential for reliable automated reagent dispensing in microfluidic workflows.

Table 3 Core functionalities of the syringe pump controller

Function	Command	Description
Valve selection	o	Rotates the valve to the specified port.
Status check	?	Returns the system status and syringe position. Status byte: ` (idle), @ (busy), or error code
Aspirate volume	P	Draws a specified volume of fluid into the syringe.
Dispense volume	D	Dispenses a specified volume of fluid from the syringe.
Move position	A	Moves the syringe plunger to an absolute position.

2.6. PC UI application

Developed using Python and PyQt5, the PC UI application is organized into four main tabs, each providing targeted control for system operation. The intuitive UI design simplifies device operation and provides precise control for reagent dispensing tasks. The script-based protocol editor further enhances automation by enabling reusable, easy-to-read commands

(Table 4). The Serial tab allows users to configure communication ports for the syringe pump and motion table. The Pump tab offers manual control of the syringe pump, including returning to the home position, selecting valves, and operating syringe movements. The Printer tab enables manual control of the 3D motion device. The Program tab supports automated execution of protocol sequences for reagent dispensing.

Table 4 Tabs in the PC UI application

Tab	Key functions
Serial	- Set communication ports - Connect/disconnect syringe pump & motion table
Pump	- Manual valve control (3-way valve) - Syringe actions: aspirate, dispense, move - Combined or separate home return - Prime: repeat aspirate/dispense between valves
Printer	- Manual 3-axis motion control - Home, Jog, Move To Position - Go To Cartridge: sequence of Z-up → X/Y move → Z-down
Program	- Load, edit, and run reagent dispensing protocols - Syntax check and execution monitoring - Line-by-line execution with live highlighting

3. Experiments and Discussion

Following the description of the overall system configuration and its key components—including the 3-axis motion table, syringe pump interface, and PC-based user application—the subsequent sections present the experimental evaluation of system performance. The evaluation focuses on positioning accuracy, dispensing accuracy, and dispensing speed, and includes a comparative analysis with previously reported methods. These experiments were conducted to validate the functionality and reliability of the developed automated liquid handling platform.

3.1. Positioning accuracy

To successfully automate reagent dispensing, the accuracy of the 3-axis motion controller is critical. Precise positioning ensures that the dispensing nozzle aligns correctly with each cartridge chamber, minimizing errors. In this study, the positioning accuracy of the motion table was evaluated using a dial gauge.

Measurement setup: A dial gauge with a resolution of 10 μm (2046SB, Mitutoyo, Japan) was mounted on the bed of the motion table using a custom jig. The gauge measured the actual displacement after commanding the table to move 1 mm along a single axis.

Procedure: The actual moved distance was determined by recording the dial gauge readings before and after the motion. Positional error was calculated by subtracting the target distance (1 mm) from the actual moved distance. Measurements were repeated 30 times for each axis, in both positive and negative directions.

Results: The results showed that the maximum positioning error across all axes remained within 31 μm (Table 5). This level of precision is sufficient to ensure accurate alignment of the dispensing nozzle with microfluidic cartridge chambers. Therefore, the system demonstrates reliable motion control performance suitable for automated reagent dispensing applications.

Table 5 Positioning accuracies (1 μm)

	+X	+Y	+Z	−X	−Y	−Z
Mean	−10.00	−8.00	3.67	−19.33	−2.33	−30.67
Std	2.62	8.05	4.90	4.50	5.68	4.50

3.2. Dispensing accuracy

The key performance metric of the automated dispensing system is the volume accuracy and consistency of the dispensed reagent. These factors directly affect the reliability of downstream diagnostic processes. To assess these characteristics, a gravimetric method was employed, as commonly used in previous studies [17, 24].

Experimental setup: Twenty empty cartridges were weighed using a precision balance (WK-3A, CAS, Korea). Then, 500 μL of distilled water was automatically dispensed into one chamber of each cartridge using the system's automated script execution feature.

Volume measurement: After dispensing, the cartridges were weighed again to determine the dispensed volume based on weight difference (Fig. 5). Each experiment was repeated twice for validation. A 1 μL volume was assumed equivalent to 1 mg of water.

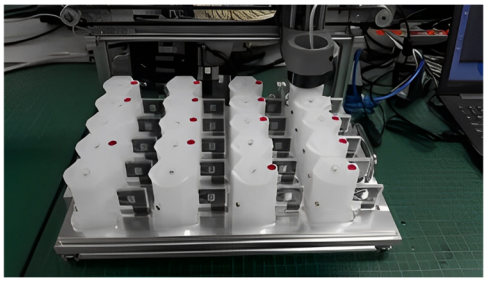

Fig. 5 The reagent dispensing experiment

Definition of accuracy and precision: To assess dispensing performance, both accuracy and precision were evaluated according to standard definitions [17, 19, 25]. Accuracy refers to systematic error and indicates how close the average of the measured values is to the target volume. It can be expressed as an absolute value (in microliters) or as a relative percentage of the target value. Precision, or random error, reflects the variability among repeated measurements. It is quantified by the standard deviation for absolute precision, and by the CV, which represents the percentage ratio of the standard deviation to the mean, for relative precision.

Results: In the case of a 500 μL target volume, the system achieved an absolute accuracy of 1.52 μL, which corresponds to a relative accuracy of 0.30%. The standard deviation of the measured volumes was 13.25 μL, yielding a relative precision of 2.64%. These values are summarized in Table 6.

Table 6 Dispensing weight (mg)

Cartridge No.	First measurement	Second measurement	Cartridge No.	First measurement	Second measurement
1	511	498	11	503	503
2	442	481	12	504	503
3	497	505	13	503	502
4	502	495	14	504	504
5	552	501	15	502	501
6	502	502	16	502	498
7	500	502	17	502	504
8	501	502	18	504	504
9	503	502	19	499	501
10	503	503	20	510	504

3.3. Dispensing speed

To achieve high-throughput automated reagent dispensing, it is essential to optimize the reagent transfer speed. To evaluate the system's performance, a time analysis was conducted during a full dispensing cycle. Specifically, the time required to dispense reagents into multiple cartridges was measured. This assessment provides insight into the system's capability for rapid and reliable fluid handling.

Experiment: Twenty cartridges were arranged on a flat tray, and 500 μL of distilled water was dispensed into each cartridge using the automated system. The total time to complete one full cycle (i.e., dispensing into all 20 cartridges) was recorded. This process was repeated 10 times to evaluate repeatability and consistency.

Results: The average time to complete one cycle was 271.3 seconds, corresponding to 13.57 seconds per cartridge (Table 7). This total time includes both the movement of the nozzle unit between cartridge positions and the actual dispensing time of the syringe pump.

Table 7 Dispensing time (s)

Trial No.	Time	Trial No.	Time
1	271	7	271
2	272	8	271
3	272	9	271
4	271	10	272
5	271	Mean	271.3
6	271	Std	0.48

3.4. Comparison with previous methods

To contextualize the performance of the developed automated reagent dispensing system, the experimental results on positioning accuracy, dispensing accuracy, and dispensing speed were compared with those reported in previous studies or observed in manual procedures.

Table 8 Comparative performance metrics

Metric	This study	Florian et al. [11]	Barthels et al. [17]	Manual operation
Positioning error (μm)	≤31	320 (X), 70 (Y,Z)	-	-
Absolute accuracy (μL)	1.52	-	0.999	-
Relative accuracy (%)	0.30	-	0.249	-
Absolute precision (μL)	13.25	-	0.866	-
Relative precision (CV, %)	2.64	-	0.215	-
Time for 20 cartridges	271.3 s (13.57 s/ea)	-	3.5 min (96 wells)	24 min (8 reagents)

Positioning accuracy: The 3-axis motion table used in this study demonstrated a maximum positioning error of 31 μm. This is significantly more precise than the system reported by Florian et al. [11], which exhibited positional errors of 320 μm, 70 μm, and 70 μm along the X, Y, and Z axes, respectively. Although a low-cost 3D printer control board and motion components were used, the system achieved relatively high positioning accuracy. This level of precision may be due to the limited range of motion required for syringe nozzle positioning and careful mechanical alignment.

Dispensing accuracy and precision: For a target volume of 500 μL, this study achieved an accuracy of 1.52 μL (0.30%) and a precision of 13.25 μL (2.64%). These values fall within the ISO 8655 limits for accuracy (≤1.6%) and precision (≤0.6%) [24], although the precision value is relatively higher due to the syringe-based actuation. Barthels et al. [17] reported slightly better precision with an accuracy of 0.999 μL (0.249%) and precision of 0.866 μL (0.215%) for a 400 μL target volume, using a pipetting robot. Nonetheless, the accuracy performance in this study is comparable, especially considering the relatively simple and cost-effective hardware used.

Dispensing speed: The automated system filled 20 cartridges (500 μL per chamber) in 271.3 seconds, averaging 13.57 seconds per cartridge. In contrast, manual dispensing of 8 reagents into 20 cartridges required approximately 3 minutes per reagent (totaling about 24 minutes). Previous automated systems for pipetting, such as those used for 96-well plate filling, reported completion times ranging from 3 minutes 30 seconds [17] to 30-45 minutes [20]. The faster dispensing speed in this study is attributed to the implementation of a syringe pump integrated with a 3-way valve, allowing rapid and repeatable volume delivery without frequent tip changes or repositioning.

Summary: Table 8 summarizes the comparative performance metrics, illustrating the advantages of the proposed system in positioning precision, dispensing accuracy, and throughput efficiency over both manual and existing automated methods. This comparison highlights that the proposed system achieves reliable performance levels for reagent dispensing in microfluidic applications, with comparable accuracy and throughput compared to previous approaches.

4. Conclusions

This study developed and validated an automated, low-cost cartridge preparation system tailored for rapid nucleic acid diagnostic workflows. The system integrates widely available components, including a 3D printer motion table, a G-code-compatible controller, and a syringe pump with a three-way valve, along with a PC-based interface for complete control. Prioritizing affordability, accuracy, and speed, the system demonstrates significant potential for practical use in laboratory and point-of-care settings. The main findings are summarized as follows:

(1) Accuracy and precision: The system achieved a volumetric accuracy of 0.30% and a precision (CV) of 2.65% when dispensing 500 μL, meeting ISO 8655 standards.

(2) Operational efficiency: A single cartridge was filled in 13.57 seconds, indicating high throughput suitable for time-sensitive applications.

(3) System flexibility: The mechanical structure, based on a CNC router frame, can be adapted to other platforms such as 3D printers or rail-based systems.

(4) Controller compatibility: Although Marlin firmware was used, the system supports any G-code-compatible controller, increasing adaptability.

(5) Novel dispensing method: The syringe pump coupled with a three-way valve enabled precise liquid handling without the need for pipette tips, reducing human error and minimizing consumable waste.

(6) Scalability and applications: The system's modular and open-source design supports expansion with components like barcode readers or heaters, extending utility to broader applications in molecular biology and diagnostics.

Nonetheless, current validation was performed using distilled water; further optimization is needed for viscous reagents such as PCR mixes. Future improvements should focus on buffer-specific handling parameters, compact pump integration, and real-time sensing for enhanced robustness and versatility.

Conflicts of Interest

The authors declare no conflict of interest.

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