



## Hardware Article

# Low-cost, open-source 3D printed antibody dispenser for development and small-scale production of lateral flow assay strips



Won Han<sup>a</sup>, Joong Ho Shin<sup>a,b,\*</sup>

<sup>a</sup> Department of Biomedical Engineering, Pukyong National University, Busan 48513, South Korea

<sup>b</sup> Industry 4.0 Convergence Bionics Engineering, Pukyong National University, Busan 48513, South Korea

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## ABSTRACT

We present a low cost, 3D printed open-source antibody dispenser that can be easily built and used for the development of lateral flow assay (LFA) strips. The fabrication of LFA strips need dispensing of antibodies or antigens in a linear fashion and commercially available dispensers typically cost from few thousands to few tens of thousands of US dollars. In this paper, an antibody dispenser was built by using 3D printed and commercially available parts, which cost no more than 30 USD. This paper presents a detailed instruction on how to assemble the printer and how to achieve a specific line width for the dispensed antibody. By using syringe needles of different gauges, line width ranging from 0.23 to 1.8 mm can be dispensed, and by varying the speed controller, the dispensing needle's speed can be varied between 2.8 and 3.8 cm/s. We demonstrate uniform dispensing of anti-C-reactive protein (CRP) antibody and anti-rabbit antibody to draw a test line and a control line, which are used for the detection of CRP. The dispenser can also be equipped with two dispensing needles to allow simultaneous dispensing of multiple reagents, which can be useful for LFA strip development.

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## Specifications table

Hardware name	Antibody Dispenser
Subject area	<ul style="list-style-type: none"> <li>Biological Sciences (e.g. Microbiology and Biochemistry) Biological Sciences (e.g. Microbiology and Biochemistry)</li> <li>Biological sample handling and preparation</li> </ul>
Hardware type	GNU General Public License (GPL) v3.0
Open Source License	\$29.63
Cost of Hardware	<a href="https://doi.org/10.17632/x2tn935zrz.1">https://doi.org/10.17632/x2tn935zrz.1</a>
Source File Repository	

\* Corresponding author.

E-mail address: [jhshin@pknu.ac.kr](mailto:jhshin@pknu.ac.kr) (J.H. Shin).

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## Hardware in context

Lateral flow assay (LFA) strips are one of the most widely used tests for point-of-care sensing and on-site detection. It can be used for many applications such as clinical analysis, food pathogen detection, environmental monitoring and veterinary medicine [1–3]. LFA is popularly used among researchers and various novel materials and methods such as plasmonic nanoparticles [4], aptamers [5], fluorescent probes [6,7] are continuously being incorporated into LFA to develop highly sensitive detection platforms that are low-cost and easy to use. Recently, due to the Corona virus disease (COVID-19) outbreak, many researchers began developing LFA tests that detect SARS-CoV-2 virus antigens [8–12], or analyze body's response after being infected from the virus [13,14]. Along with the FDA approval of Quidel's Sofia SARS Antigen FIA kit, many companies also started producing their own LFA test kits related to COVID-19 as well, and their performances are being evaluated in universities and hospitals [15,16]. These cheap and quick method of testing, if developed and used correctly, is expected to help manage the disease [17]. Such trend in both the academia and the industry shows that there is a need of an affordable antigen or antibody dispenser, and an open-source dispenser has the potential to accelerate the research and development of LFA in both realms.

The fabrication of the LFA strip involves dispensing of antibody reagents or antigen reagents across the surface of porous substrates in a form of a straight line, which typically require expensive instruments. The dispensed antibodies are dried, and then the substrates are cut perpendicularly to form thin strips. The dispensers used in mass production of LFA strips can cost tens of thousands of dollars because of the complexities that arise from their ability to dispense or spray small amount of reagents, and the ability to precisely control the dispenser head's position. Such instruments are difficult to be accessed by users whose work does not involve or require mass production. For researchers and lab scale production for research and feasibility tests, simpler form of dispensers are available at lower cost from companies such as Claremont BioSolutions, LLC, Rega Biotechnology Inc, Advanced Microdevices Pvt. Ltd. Typical form of their dispensers use syringe needles that moves across stationary nitrocellulose (NC) membrane. Small amount of antibody reagent is stored inside of the needle, and the reagent is dispensed passively by the wicking force caused by the pores of the NC membranes. More complicated form of the dispensers attach syringe pumps to the needles, and actively pump liquid out of the needles. The commercially available dispensers cost from \$4,500 to little over \$9,000, depending on the additional features and options.

In academia, research labs often use pipettes and dispense 1–2  $\mu\text{L}$  of antibodies onto the NC membrane due to the lack of accessibility to antibody dispensers. The method is good enough to validate new sensing modalities and concept validations, however, the resulting test signals appear as a circular or semicircular shape with uneven signal intensity [18–20]. Furthermore, dispensing performed by experimenters may result in variation of signal intensity because of inconsistent dispensing. Few research groups attempted to dispense antibodies in a consistent manner. Choi et al. demonstrated the use of fountain pens and a pen holder that helps users to glide the pen over NC membrane [21], and Credou et al. demonstrated the use of a commercially available inkjet printer to print antibody patterns on a cellulose substrate [22]. However, it is important to develop a system that eliminates human variation, and also be economical and easily accessible through open-sourcing.

In this paper, we present a low-cost, open-source antibody dispenser that can be easily fabricated in a research lab. The dispenser uses 3D printed parts, commercially available electronics and syringe needle tips. Instead of using an external pump for reagent dispensing, the dispenser passively dispenses reagents via the capillary force of the NC membrane as the needle tip comes in contact with the membrane. The assembled device costs as little as \$29.63 excluding the shipping costs of the parts, and 3D printing all the parts required using a single 3D printer requires about 15 h at most. After the parts are printed and ready for assembly, it takes an undergraduate university student 1 h to assemble, which includes uploading the source code into the Arduino board. User can easily control the speed of dispensing, exchange needle tips, and add additional needle tip holders for multiple reagent dispensing. As a demonstration, we use the antibody dispenser to fabricate a LFA strip and perform detection of human C-reactive protein (CRP) from phosphate-buffered saline (PBS). To the best of our knowledge, this is the first paper to report an open-source hardware that can dispense bioreagents for the development of LFA strips.

## Hardware description.

### *Overall description of the dispenser*

Conventional antibody dispensers manufactured by the three companies mentioned above operates by dragging dispensing needles across the surface of an NC membrane. This limits the length of the membrane that can be treated with antibodies, hence, we designed the dispenser so that the membrane moves instead of the needle, while the needle is stationary. The trajectory of the NC membrane is guided by a 0.5 mm slit, which allows the NC membrane to slide without getting out of the track. While the membrane slides, a needle with antibody reagent stored inside makes a contact with the membrane's surface, and the reagent is dispensed passively. This allows continuous feeding of the NC membrane and dispensing of antibody reagents.

### *The wheels*

Two wheels attached with o-rings are used to slide the membrane across the platform. The wheels are turned by motors with a built-in reduction gear housing, and o-rings are used to provide friction. It is important to press the NC membrane hard enough to provide friction force and traction, but not to a point where the force damages NC membrane and cause

the pores to collapse. Thus a mechanism was employed to be able to easily adjust the height of the wheels and easily control the friction force. The dispenser has a rotary switch that can vary the speed of the wheel's rotation, which in turn controls the velocity of the membrane. For easy replication of the open-source device, Arduino Uno was used to control the dispenser.

### *Passive dispensing*

NC membrane's pores cause a capillary force, which wicks the liquid from the needle's tip and wets the membrane. Exploiting this phenomenon allows the dispenser to operate without using additional pumping mechanisms such as syringe pumps or pneumatic pumps. It is important to note that using membranes of different pore sizes may result in different line width of the dispensed reagent. Smaller pores may have larger capillary force, but the high fluidic resistance of the small pores may hinder reagent dispensing. On the contrary, larger pores may have smaller capillary force, but more reagent may be dispensed per length due to lower fluidic resistance. Thus, users should observe and calibrate the desirable line width before dispensing reagents.

### *The dispensing head*

The dispensing head used in this study is a syringe needle, which is commercially available, and can be easily attached and detached from the needle holder. This allows users to use needles with different diameters according to their needs. Additionally, multiple needle holders can be installed to the dispenser, which allows simultaneous dispensing of multiple antibody reagents. This can, for example, allow simultaneous dispensing of capture antibodies and control antibodies, which are essential for LFA test strips.

### *Possible applications*

- Researchers can use this device to dispense antibody in a linear form to help develop LFA test strips for concept validation and feasibility tests.
- The dispenser can be used to dispense antibodies to develop LFA tests that operate based on sandwich assay formats.
- The dispenser also can be used to dispense antigens to develop LFA tests that operate based on competitive assay formats.
- The low-cost device can be easily made and be applied in research labs and small companies for cost as low as \$29.63.

## **Design files**

### *Design files summary*

Design file name	File type	Open source license	Location of the file
Arduino code.txt	Code (txt)	GNU GPL v3	<a href="https://doi.org/10.17632/x2tn935zrz.1">https://doi.org/10.17632/x2tn935zrz.1</a>
arm_1.stl	CAD (STL)	GNU GPL v3	<a href="https://doi.org/10.17632/x2tn935zrz.1">https://doi.org/10.17632/x2tn935zrz.1</a>
arm_2.stl	CAD (STL)	GNU GPL v3	<a href="https://doi.org/10.17632/x2tn935zrz.1">https://doi.org/10.17632/x2tn935zrz.1</a>
arm_fastener.stl	CAD (STL)	GNU GPL v3	<a href="https://doi.org/10.17632/x2tn935zrz.1">https://doi.org/10.17632/x2tn935zrz.1</a>
back_cover.stl	CAD (STL)	GNU GPL v3	<a href="https://doi.org/10.17632/x2tn935zrz.1">https://doi.org/10.17632/x2tn935zrz.1</a>
front_cover.stl	CAD (STL)	GNU GPL v3	<a href="https://doi.org/10.17632/x2tn935zrz.1">https://doi.org/10.17632/x2tn935zrz.1</a>
main_body_bottom.stl	CAD (STL)	GNU GPL v3	<a href="https://doi.org/10.17632/x2tn935zrz.1">https://doi.org/10.17632/x2tn935zrz.1</a>
main_body_top.stl	CAD (STL)	GNU GPL v3	<a href="https://doi.org/10.17632/x2tn935zrz.1">https://doi.org/10.17632/x2tn935zrz.1</a>
motor_holder.stl	CAD (STL)	GNU GPL v3	<a href="https://doi.org/10.17632/x2tn935zrz.1">https://doi.org/10.17632/x2tn935zrz.1</a>
needle_holder.stl	CAD (STL)	GNU GPL v3	<a href="https://doi.org/10.17632/x2tn935zrz.1">https://doi.org/10.17632/x2tn935zrz.1</a>
pin.stl	CAD (STL)	GNU GPL v3	<a href="https://doi.org/10.17632/x2tn935zrz.1">https://doi.org/10.17632/x2tn935zrz.1</a>
wheel.stl	CAD (STL)	GNU GPL v3	<a href="https://doi.org/10.17632/x2tn935zrz.1">https://doi.org/10.17632/x2tn935zrz.1</a>

**Bill of materials***Bill of materials*

Designator	Component	Number	Cost per unit -USD	Total cost- USD	Source of materials	Material type
Arduino board	Aduino Uno	1	\$23	\$23	<a href="https://www.amazon.com/Arduino-A000066-ARDUINO-UNO-R3/dp/B008GRTSV6/ref= sr_1_3?dchild=1&amp;keywords=arduino&amp;qid=1598532004&amp;sr=8-3=">https://www.amazon.com/Arduino-A000066-ARDUINO-UNO-R3/dp/B008GRTSV6/ref= sr_1_3?dchild=1&amp;keywords=arduino&amp;qid=1598532004&amp;sr=8-3=</a>	Electronic
Motor driver	L298N	2	\$0.83	\$1.66	<a href="https://www.aliexpress.com/item/32845457866.html?spm=a2g0o.productlist.0.0.3ab74858JcIDFF&amp;algo_pvid=2eb09d01-08a1-4e69-bf24-c21e8a9f830b&amp;algo_expid=2eb09d01-08a1-4e69-bf24-c21e8a9f830b-6&amp;btsid=0ab6fb8815985320992542709e9ece&amp;ws_ab_test=searchweb0_0,searchweb201602_,searchweb201603_">https://www.aliexpress.com/item/32845457866.html?spm=a2g0o.productlist.0.0.3ab74858JcIDFF&amp;algo_pvid=2eb09d01-08a1-4e69-bf24-c21e8a9f830b&amp;algo_expid=2eb09d01-08a1-4e69-bf24-c21e8a9f830b-6&amp;btsid=0ab6fb8815985320992542709e9ece&amp;ws_ab_test=searchweb0_0,searchweb201602_,searchweb201603_</a>	Electronic
Variable resistor	WH148	1	\$0.33	\$0.66	<a href="https://www.aliexpress.com/item/33048389515.html?spm=a2g0o.productlist.0.0.1a326dc79Yer9G&amp;algo_pvid=200ab051-437f-4569-9815-5e603b3f06be&amp;algo_expid=200ab051-437f-4569-9815-5e603b3f06be-5&amp;btsid=0ab50a5715985329123711876ef560&amp;ws_ab_test=searchweb0_0,searchweb201602_,searchweb201603_">https://www.aliexpress.com/item/33048389515.html?spm=a2g0o.productlist.0.0.1a326dc79Yer9G&amp;algo_pvid=200ab051-437f-4569-9815-5e603b3f06be&amp;algo_expid=200ab051-437f-4569-9815-5e603b3f06be-5&amp;btsid=0ab50a5715985329123711876ef560&amp;ws_ab_test=searchweb0_0,searchweb201602_,searchweb201603_</a>	Electronic
LCD panel	16x2 I2C LCD module	1	\$1.41	\$1.41	<a href="https://www.aliexpress.com/item/32890910334.html?spm=a2g0o.productlist.0.0.709a6965EosMWL&amp;algo_pvid=dc6af4a3-862e-4f00-8e3e-4d285d30d413&amp;algo_expid=dc6af4a3-862e-4f00-8e3e-4d285d30d413-6&amp;btsid=0ab6f82315985331389731117e5534&amp;ws_ab_test=searchweb0_0,searchweb201602_,searchweb201603_">https://www.aliexpress.com/item/32890910334.html?spm=a2g0o.productlist.0.0.709a6965EosMWL&amp;algo_pvid=dc6af4a3-862e-4f00-8e3e-4d285d30d413&amp;algo_expid=dc6af4a3-862e-4f00-8e3e-4d285d30d413-6&amp;btsid=0ab6f82315985331389731117e5534&amp;ws_ab_test=searchweb0_0,searchweb201602_,searchweb201603_</a>	Electronic
O-ring	Rubber O-ring (CS 3.5 mm, OD 41.5 mm)	4	\$0.033	\$0.132	<a href="https://www.amazon.com/ABN-Assortment-419-Piece-Metric-Rings/dp/B00KC3CUJE/ref=pd_rhf_se_s_sspa_dk_rhf_search_pt_sub_0_5/136-7149333-2002339?_encoding=UTF8&amp;pd_rd_i=B00KC3CUJE&amp;pd_rd_r=4e492b47-35a1-416b-9388-0f017446716d&amp;pd_rd_w=2ICSn&amp;pd_rd_wg=UTDuu&amp;pf_rd_p=35464220-1a6e-4035-aa4f-d89021920d73&amp;pf_rd_r=7WV4GR8FBCEXNT13HHYM&amp;psc=1&amp;refRID=7WV4GR8FBCEXNT13HHYM&amp;spLa=ZW5jcnlwdGVkUXVhbGlmaWVyPUFISFU5QloxVUtKSk8mZW5jcnlwdGVkSWQ9QT A0MTY2NjUxVjdIRzU2Q1ExT1o1JmVuY3J5cHRIZEFkSWQ9QTA2OTUyOTNOWDJWSVIQM U9ZVkmMmd2lkZ2V0TmFtZT1zcF9yaGZfc2VhcmNoJmFjdGlvbj1jbGlja1JlZGlyZWNOJmRvTm90TG9nQ2xpY2s9dHJ1ZQ==">https://www.amazon.com/ABN-Assortment-419-Piece-Metric-Rings/dp/B00KC3CUJE/ref=pd_rhf_se_s_sspa_dk_rhf_search_pt_sub_0_5/136-7149333-2002339?_encoding=UTF8&amp;pd_rd_i=B00KC3CUJE&amp;pd_rd_r=4e492b47-35a1-416b-9388-0f017446716d&amp;pd_rd_w=2ICSn&amp;pd_rd_wg=UTDuu&amp;pf_rd_p=35464220-1a6e-4035-aa4f-d89021920d73&amp;pf_rd_r=7WV4GR8FBCEXNT13HHYM&amp;psc=1&amp;refRID=7WV4GR8FBCEXNT13HHYM&amp;spLa=ZW5jcnlwdGVkUXVhbGlmaWVyPUFISFU5QloxVUtKSk8mZW5jcnlwdGVkSWQ9QT A0MTY2NjUxVjdIRzU2Q1ExT1o1JmVuY3J5cHRIZEFkSWQ9QTA2OTUyOTNOWDJWSVIQM U9ZVkmMmd2lkZ2V0TmFtZT1zcF9yaGZfc2VhcmNoJmFjdGlvbj1jbGlja1JlZGlyZWNOJmRvTm90TG9nQ2xpY2s9dHJ1ZQ==</a>	Polymer

a (continued)

Designator	Component	Number	Cost per unit -USD	Total cost- USD	Source of materials	Material type
Breadboard	Mini breadboard	1	\$0.8325	\$0.8325	<a href="https://www.amazon.com/dp/B07KG8VW21/ref=sspa_dk_detail_1?psc=1&amp;spLa=ZW5jcjnlwdGVkUXVhbGlmaWVyYyPUBFKMTdZU1A1RDVZNkcmZW5jcjnlwdGVkSWQ9QTAxMDUwMDZVRVBVMzdaQ0NIS0kmZW5jcjnlwdGVkQWRJZD1BMDA4ODQyME9VT0g4Uk5OT0NZWCZ3aWRnZXROYW1lPXNwX2RldGFpbDlmYWN0aW9uPWNsaWNRUmVkaXJlY3QmZG90b3RMb2dDbGljaz10cnVl">https://www.amazon.com/dp/B07KG8VW21/ref=sspa_dk_detail_1?psc=1&amp;spLa=ZW5jcjnlwdGVkUXVhbGlmaWVyYyPUBFKMTdZU1A1RDVZNkcmZW5jcjnlwdGVkSWQ9QTAxMDUwMDZVRVBVMzdaQ0NIS0kmZW5jcjnlwdGVkQWRJZD1BMDA4ODQyME9VT0g4Uk5OT0NZWCZ3aWRnZXROYW1lPXNwX2RldGFpbDlmYWN0aW9uPWNsaWNRUmVkaXJlY3QmZG90b3RMb2dDbGljaz10cnVl</a>	Electronic
Motor	6V Reduction gear motor	2	\$0.97	\$1.94	<a href="https://www.aliexpress.com/item/4000832128439.html?spm=a2g0o.productlist.0.0.728b1ee4rS7XGc&amp;algo_pvid=7be13338-d09f-41ee-8a32-2a884f3d6943&amp;algo_expid=7be13338-d09f-41ee-8a32-2a884f3d6943-5&amp;btsid=0ab6d69f15985338860038016e621c&amp;ws_ab_test=searchweb0_0,searchweb201602_,searchweb201603_">https://www.aliexpress.com/item/4000832128439.html?spm=a2g0o.productlist.0.0.728b1ee4rS7XGc&amp;algo_pvid=7be13338-d09f-41ee-8a32-2a884f3d6943&amp;algo_expid=7be13338-d09f-41ee-8a32-2a884f3d6943-5&amp;btsid=0ab6d69f15985338860038016e621c&amp;ws_ab_test=searchweb0_0,searchweb201602_,searchweb201603_</a>	Electronic
Button					<a href="https://www.aliexpress.com/item/33004148697.html?spm=a2g0o.productlist.0.0.4ec626e0M8C0gr&amp;algo_pvid=c7b63112-a98f-4c7f-8dc1-2732526cb1a2&amp;algo_expid=c7b63112-a98f-4c7f-8dc1-2732526cb1a2-49&amp;btsid=0be3743b15986029727632547e830d&amp;ws_ab_test=searchweb0_0,searchweb201602_,searchweb201603_">https://www.aliexpress.com/item/33004148697.html?spm=a2g0o.productlist.0.0.4ec626e0M8C0gr&amp;algo_pvid=c7b63112-a98f-4c7f-8dc1-2732526cb1a2&amp;algo_expid=c7b63112-a98f-4c7f-8dc1-2732526cb1a2-49&amp;btsid=0be3743b15986029727632547e830d&amp;ws_ab_test=searchweb0_0,searchweb201602_,searchweb201603_</a>	Electronic

Total cost to build a single dispenser: \$29.63

## Build instructions

### Electronics

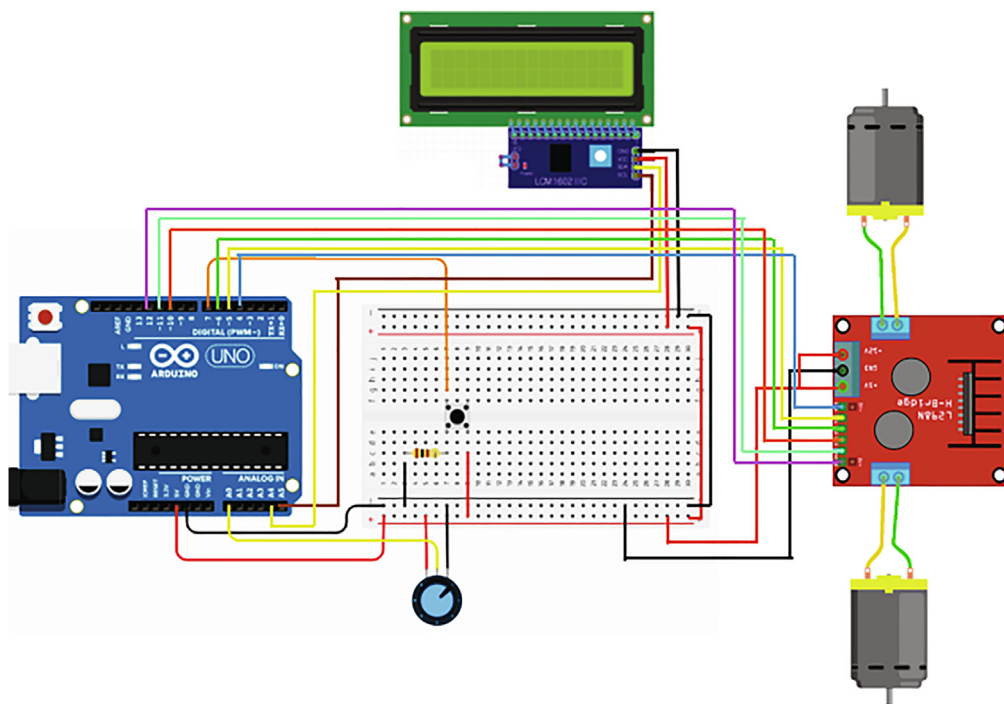
As shown in Fig. 1, Connect the variable resistor to A0, 5 V and GND pin of the Arduino board. Connect the LCD panel's GND, VCC, SDA and SCL to Arduino board's GND, 5 V, A4, and A5 respectively. Connect motor driver's IN2, IN1 and ENA to Arduino board's digital pin 4, 5 and 6 respectively. Then, connect the motor driver's IN3, IN4 and ENB to Arduino board's digital pin 10, 11 and 12. Connect Arduino's 5 V and GND to the motor driver', and connect the push button to Arduino board's digital pin 7. Connect the Arduino board to the PC and upload code.ino file. Remove the cover of CON5 pin (Fig. 2). Connect the motor driver's 12 V terminal to 5 V terminal so that the driver can operate by using the 5 V voltage source from Arduino. Use a male to male wire to connect one of the motors to output 1 and 2 of the motor driver, and connect the other motor to output 3 and 4 of the motor driver.

### Wheel assembly

Download and 3D print wheel.stl and motor\_holder.stl file. Print two wheel barrels. The printed wheel barrel should have a groove at each end so that the o-rings can stay in their place (Fig. 3A). Place two o-rings for each barrel and make sure that the o-ring sits in the groove (Fig. 3B, C). Attach the wheel assembly by inserting the motor's shaft into the hole of the wheel barrel (Fig. 3D). Lastly, insert the motor into the motor holder (Fig. 3E).

### Dispenser assembly

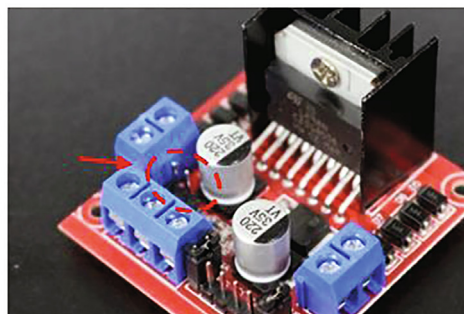
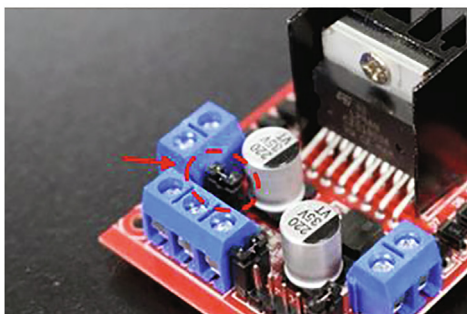
Download and 3D print main\_body\_bottom.stl, main\_body\_top.stl, back\_cover.stl, front\_cover.stl, pin.stl, needle\_holder, arm\_1.stl, arm\_2.stl, arm\_fastener.stl files and remove the supports. Place the main board into the bottom part of the body in a manner that allows the LCD, variable resistor and push button to be fitted in the windows shown in the front of the body (Fig. 4), and then close the main body by assembling the top part (Fig. 5). Slide the motor holder into the grooves of the post



**Fig. 1.** Wiring diagram of the electronic components used for making the antibody dispenser. For prototyping, Arduino Uno and breadboard are used. The motors are connected to a motor driver, whose speed is controlled by a variable resistor.

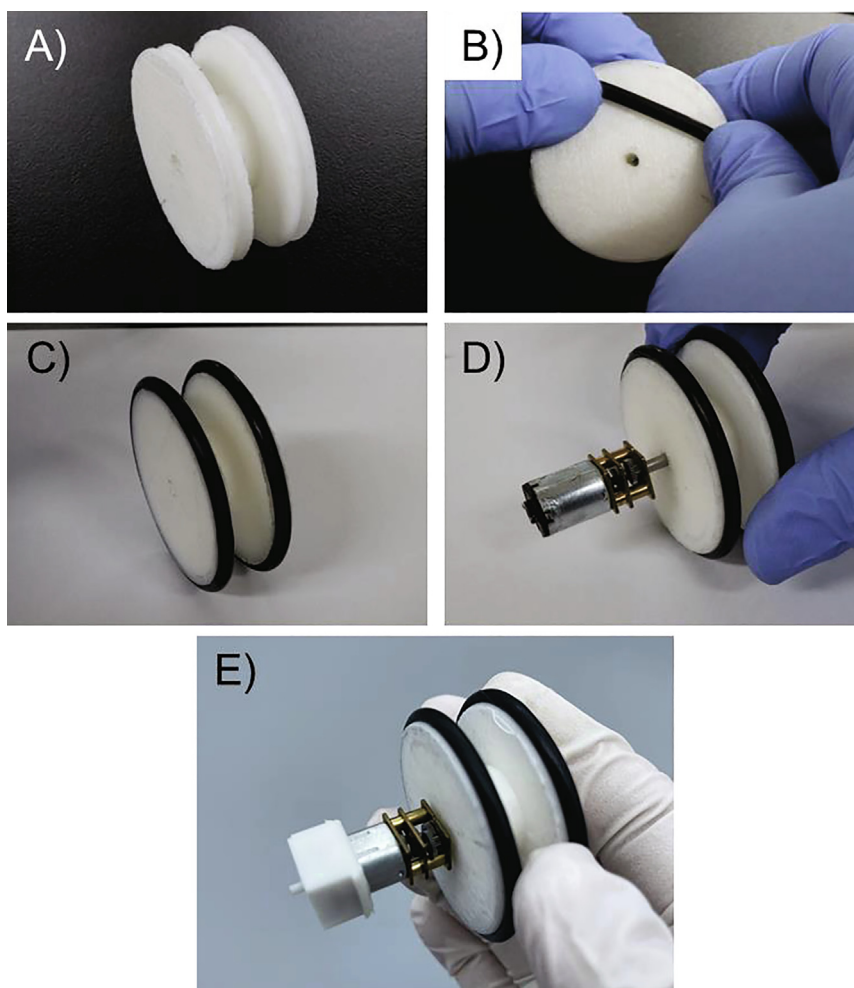
Before removing  
CON5 cover

After removing  
CON5 cover

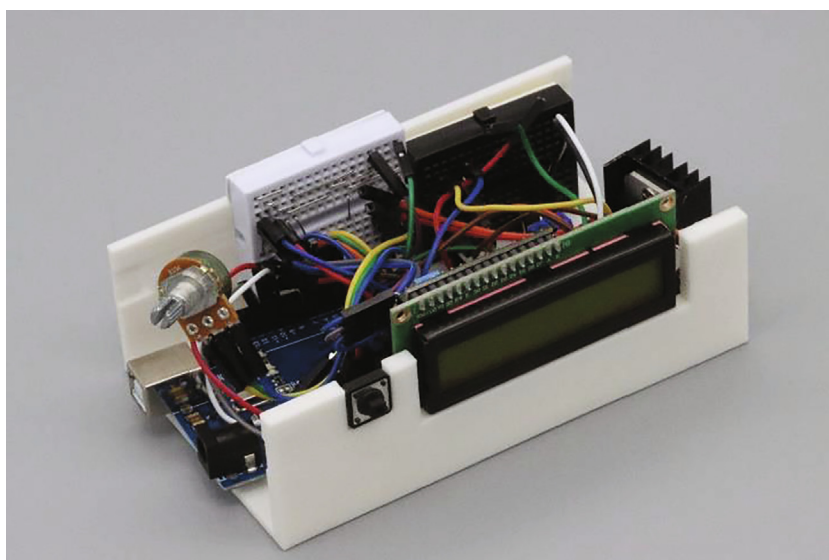


**Fig. 2.** Photo of the motor driver before removing CON5 cover (left) and after removing CON5 cover (right).

(Fig. 6A) and connect the wires to the motor's electrodes (Fig. 6B). To assemble a wheel height adjustment unit, assemble arm\_1, arm\_2, and arm\_fastener by inserting an M4 × 10 mm screw into their hole (Fig. 7A). Place an M4 nut into the slot of arm\_fastener (Fig. 7B), and fasten them using a nut (Fig. 7C). Assemble the wheel height adjustment unit with the motor holder by inserting the motor holder's pin into one the hole of the wheel height adjustment unit (Fig. 8). Repeat the assembly for the other wheel. The wheel height adjustment unit was designed to easily adjust the height of the wheel barrel with respect to the NC membrane (Fig. 9). Once the user sets the height that allows robust passing of NC membrane without any noticeable damage to the membrane such as crushing or tearing, the user can fasten the holder to maintain the wheel's height (Fig. 4G). If the height needs to be adjusted, the user can simply unfasten the holder, adjust the height again, and then fasten the holder. Bend the syringe needle by approximately 45° off axis and insert it into the needle holder (Fig. 10A). Insert the pin through the hole placed at the top part of the body and also through the needle holder (Fig. 10B). Use the front cover and back cover to close the sides of the device (Fig. 11A) and connect the cable to power the device (Fig. 11B). It is important



**Fig. 3.** A) 3D printed wheel with grooves. B) Photo of o-ring being places in the groove. C) Wheel with two o-rings assembled. D) Motor's shaft is fitted into the wheel's axis. E) Motor is fitted into the motor holder.



**Fig. 4.** Main board and electronic parts placed in the bottom part of the main body (main\_body\_bottom.stl).

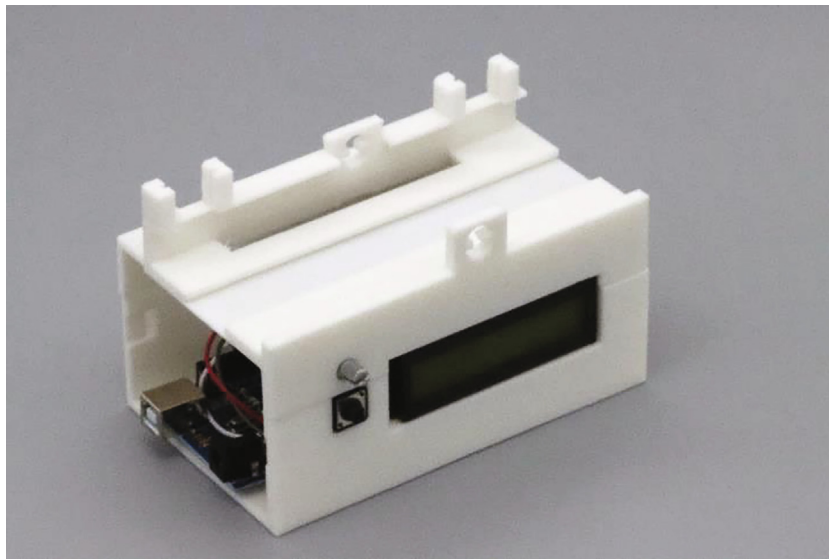


Fig. 5. Top part (main\_body\_top.stl) is assembled to encase the electronics.

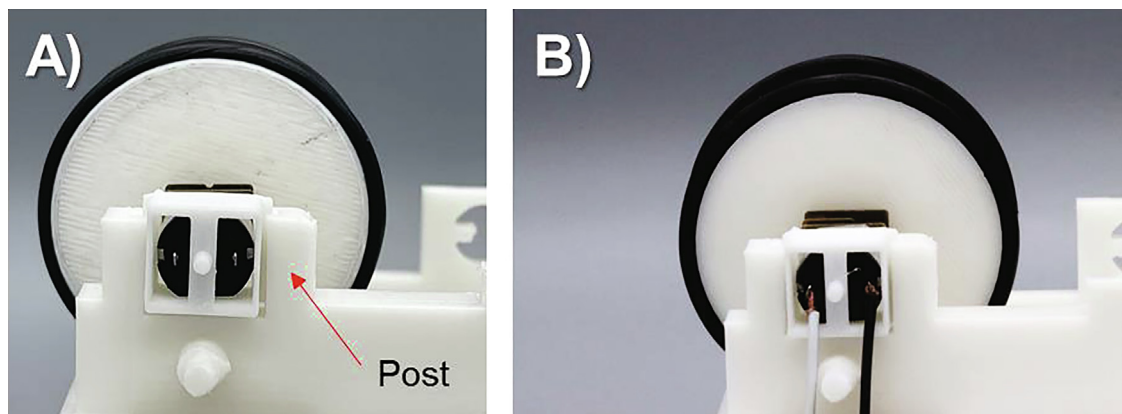


Fig. 6. A) Motor holder is slid into the post's grooves. B) Wires are connect to the motor's electrodes.



Fig. 7. Wheel height adjustment unit. A) arm\_1 and arm\_2 connected by a screw. B) Screw is held in place by using arm\_fastener (indicated with red box and red arrow). C) Pieces are fastened by using an M4 nut. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to have a small gap between the hole of the needle holder (Fig. 10A) and the pin (Fig. 10B) so that the needle holder is free to rotate about the axis of the pin. This allows the tip of the needle to rest on the rail platform initially, and the NC membrane to lift the needle and slide underneath it. As the NC membrane slides, the weight of the needle holder gently pushes the needle tip onto the membrane to ensure a contact.



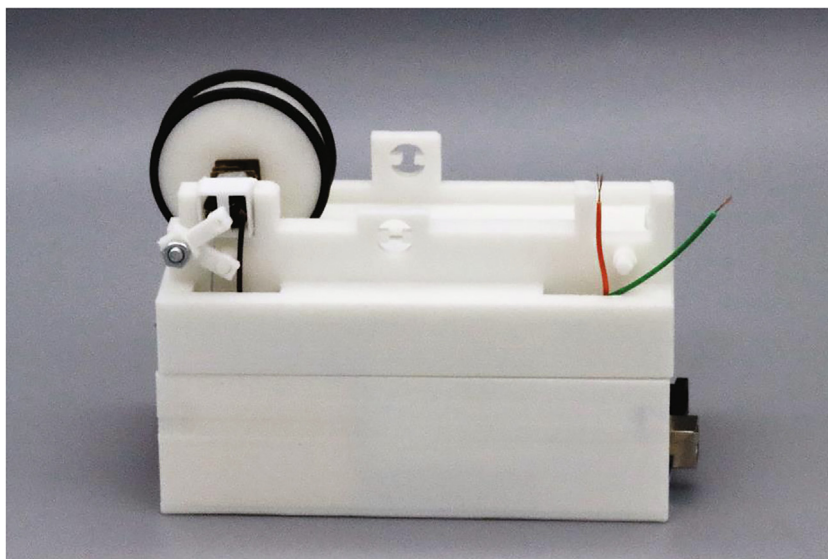


Fig. 8. Wheel height adjustment unit is assembled with the motor holder.

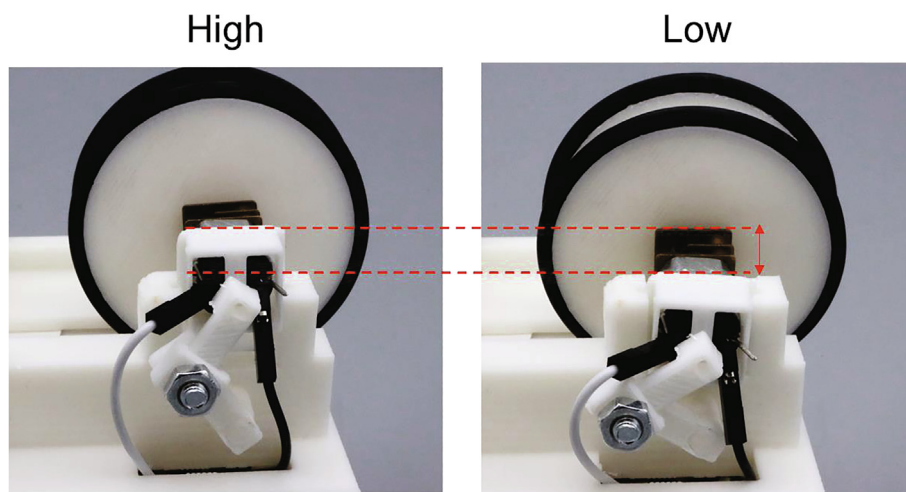
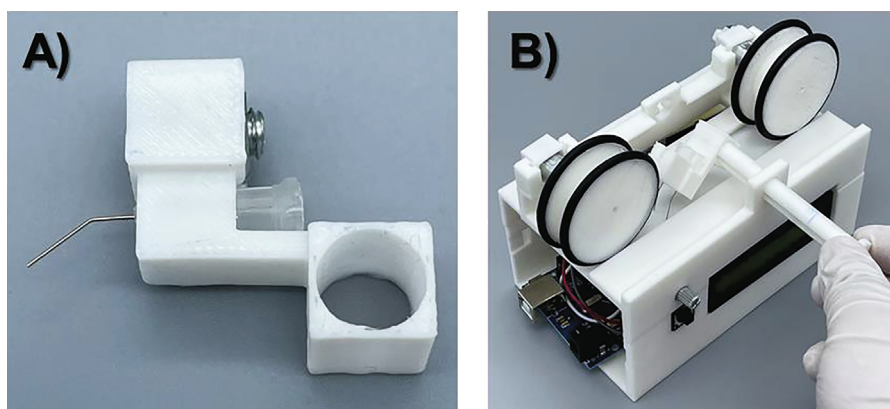


Fig. 9. Wheel height adjustment unit's joint angle can be adjusted and fastened to set the proper height required for passing NC membranes.

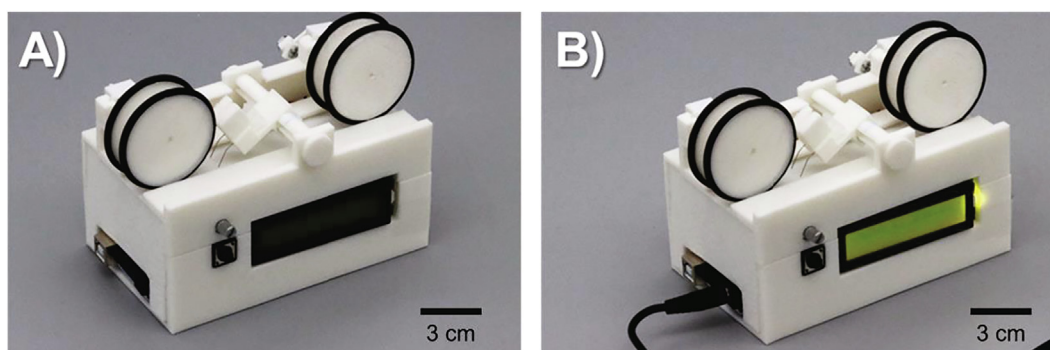
## Operation instructions

### Operation steps

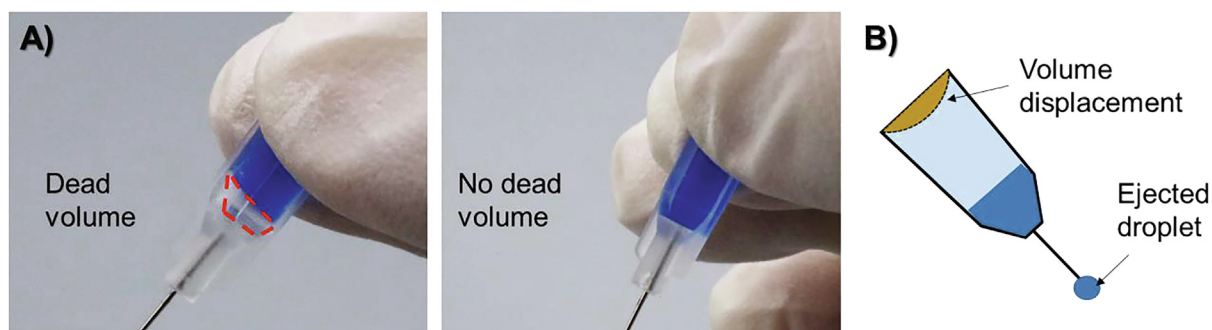
- 1) Pipette antibody (or other reagents) into the bottom of the syringe needle. Be careful not to trap bubbles near the narrow part of the dead space (Fig. 12A)
- 2) Press the open end of the syringe needle with the user's thumb until a droplet is formed at the needle tip by the volume displaced by the pressed thumb to prime the empty needle (Fig. 12B)
- 3) Rest the syringe needle on the rail of the dispenser and adjust the value of the variable resistor (between 0 and 255) shown on the LCD panel according to the user's preference (Fig. 13). The resistor's value determines the speed of NC membrane.
- 4) After setting the speed, press the button located under the variable resistor to start the motor.
- 5) Insert the NC membrane at the entrance of the rail until the front of the membrane is placed between the wheel 1 and the bottom of the rail platform (Fig. 14)
- 6) Be careful not to let any contaminants such as dirt, dust or any other liquid come in contact with the NC membrane.
- 7) After dispensing, flush the needle with 70% ethanol using a syringe, and flush with air to dry.



**Fig. 10.** A) Needle is assembled with the needle holder. B) Pin being inserted into the main body and the needle holder.



**Fig. 11.** A) Front and back cover is used to close the device. B) Connect the power cable.



**Fig. 12.** A) Photo of reagent filled in the syringe needle with a dead volume (left) and properly filled syringe needle (right). B) Schematic of finger pressed at the top of the needle to displace air volume and eject reagent out of the needle.

### Operation tips

The reagents should be dispensed onto the membrane immediately after being loaded into the needle tip, and storing the reagents in the needle tip at room temperature should be avoided in order to avoid thermal degradation of the biomolecules. It is necessary to prime the needle as described in Fig. 12. This is crucial to ensure a contact between the liquid and the membrane and initiate capillary driven dispensing of the reagents. Additionally, it is advisable to bend the needle as shown in Fig. 10A. This helps the reagents from the needle tip to contact the membrane. The bending angle does not have to be perfectly 45 deg, but the angle should be such that the cross section of the needle tip is nearly perpendicular to the membrane. Care should be taken to avoid a direct contact between the o-ring of the wheel and the dispensed antibody line. The

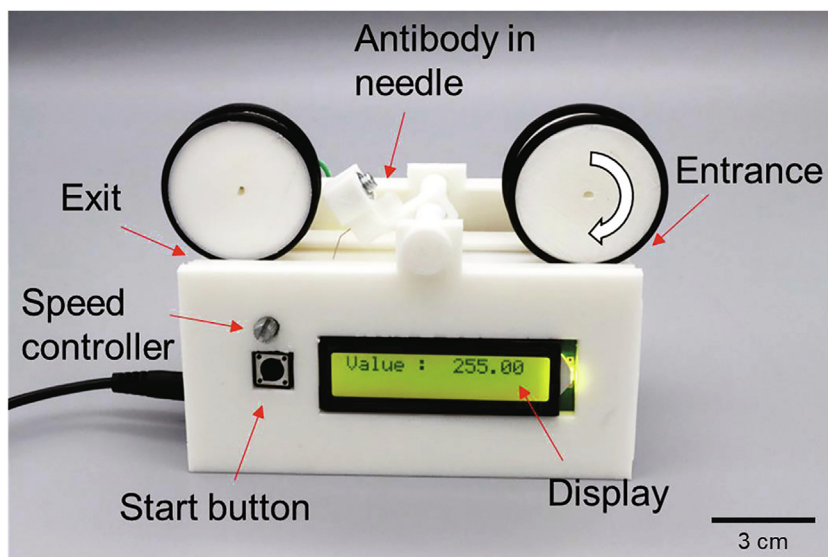


Fig. 13. Photo of the antibody dispenser ready for operation.

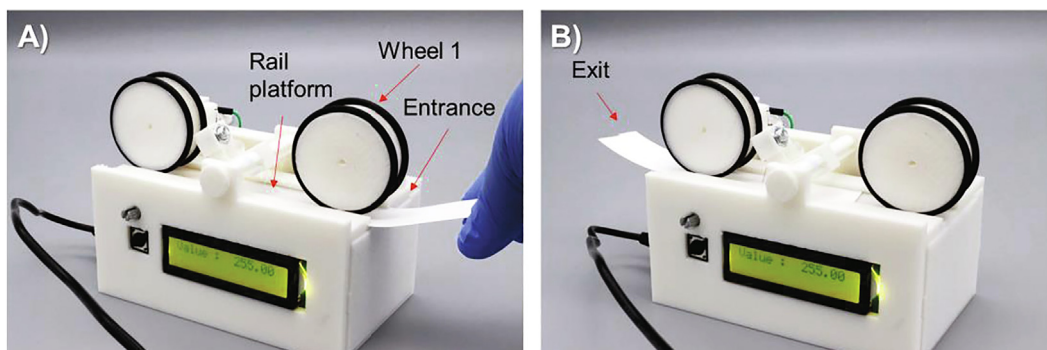


Fig. 14. A) NC membrane being inserted into the antibody dispenser's entrance and B) NC membrane exiting the dispenser.

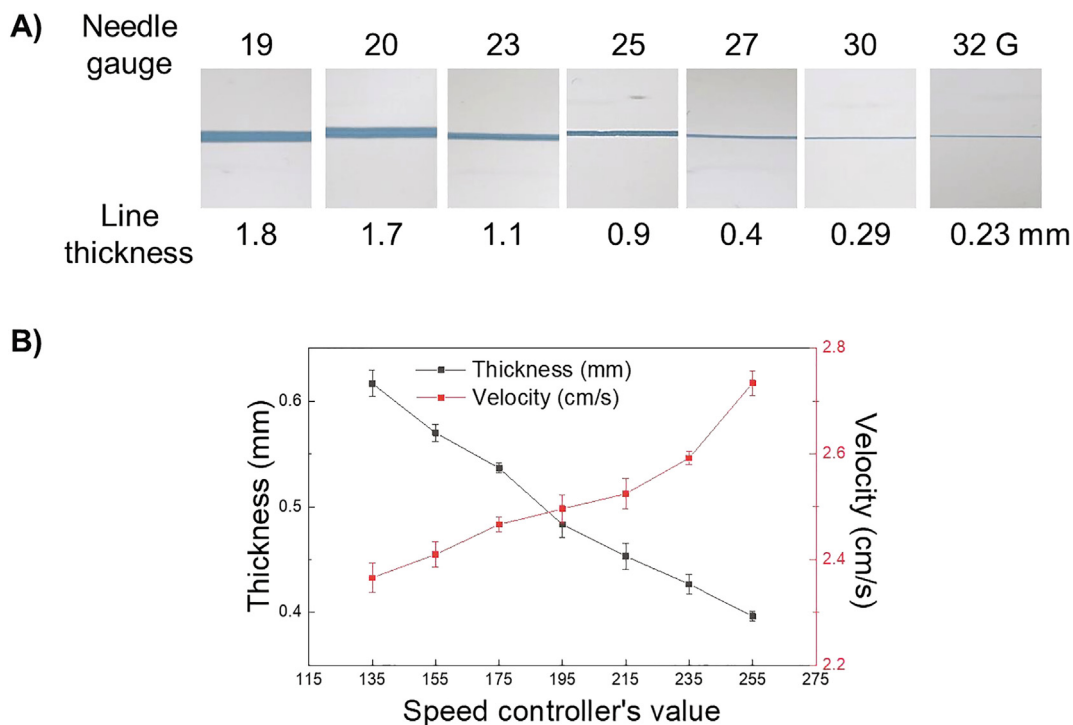
dispensed antibody may wet the wheel if it comes in contact with the wheel immediately after being dispensed. This can be easily avoided by positioning the needle holder in between the two o-rings.

## Validation and characterization

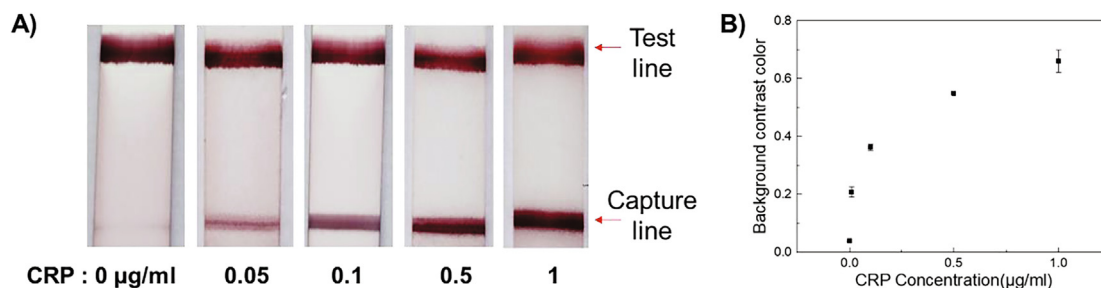
### Characterization of the dispenser

The antibody is dispensed passively onto the NC membrane as the membrane passes and the pore's capillary force wicks the liquid from the needle tip, resulting in a line of dispensed liquid. Thus, the diameter of the needle and the membrane's travel velocity are two operational parameters that determine the width of the line. The flow resistance through the needle is inversely proportional to the diameter of the needle, thus larger diameter would theoretically result in thicker line, and vice versa. We compared three needles of different gauges (19, 20, 23, 25, 27, 30 and 32 G) to determine the effect of needle diameter on the line width. Fig. 15A shows that larger needle diameter results in thicker line as expected, and the result indicates that the antibody dispenser is compatible with wide range of commercially available syringe needles.

The amount of liquid wicked into the membrane is proportional to the duration of time the needle made contact with the membrane. This means that lower velocity of the membrane allows the needle to spend long time contacting the membrane, which in turn results in more liquid dispensed and a thicker line. Thus we varied the speed controller's value from 135 to 255 (arbitrary unit) and measured the travel velocity of the membrane and the corresponding dispensing profile. Fig. 15B shows that as the speed controller's value increases, the velocity of the membrane also increases. Using 27 G needle, we observed the width of the dispensed liquid line according to the membrane velocity. The result shows that the line width varies about 0.2 mm depending on the speed controller's value. This indicates that the thickness of the dispensed reagent can be



**Fig. 15.** A) Photos showing dispensed blue dye using different needle gauges and their measured thickness showing at the bottom. B) Graph showing the thickness of the dispensed blue line and the NC membrane's linear velocity versus speed controller's value (the speed of the dispenser's wheels). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 16.** A) Signals appear at the capture line of LFA strips after loading samples containing CRP of different concentrations. B) The signal intensity increases with increasing CRP concentration.

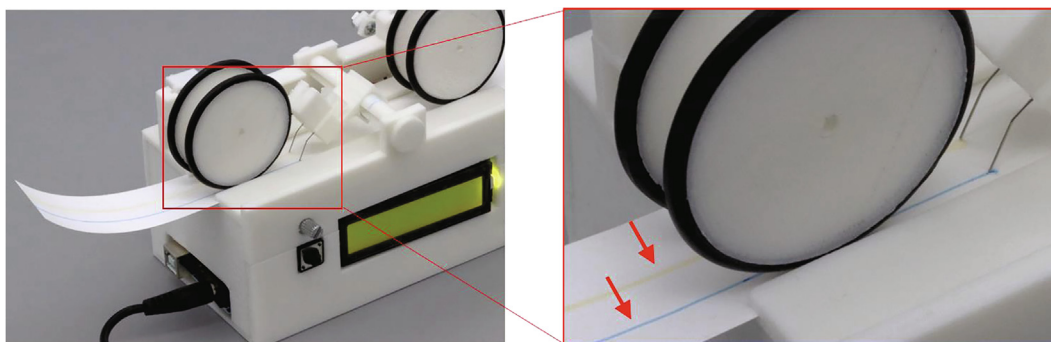
controlled both by the needle gauge and the speed controller's value. Furthermore, the minimum volume of the reagents that can be handled in order to successfully dispense antibody is 1.0 µL, which can print 1.8 cm using a 30 G needle at speed controller value set at 255. Additionally, the maximum volume that can be stored in the needle is 130 µL.

#### Demonstration of lateral flow assay strip fabrication

The dispenser was used to dispense antibodies for lateral flow assay (LFA) strip fabrication. As a demonstration, human C-reactive protein (CRP) detection LFA strip was fabricated, and the dispenser was used to dispense capture line and a control line, which capture CRP and rabbit IgG respectively. 27 G needle was used to immobilize antibodies on the NC membrane. Fig. 16A shows that the signal appears at the test line for all strips and the capture line's intensity increases as the concentration of CRP in PBS increases (Fig. 16B).

#### Simultaneous printing of multiple reagents

Fabrication of LFA strip always require immobilization of at least two different antibodies: one for the target analyte, and the other as a control line. The control line's antibody binds with the antibody that is conjugated with the gold nanoparticle,



**Fig. 17.** Antibody dispenser printing two reagents simultaneously using two needle holders (left) and a close-up view showing yellow and blue dye being printed from two needles (right). The dispensed dye is indicated by red arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

so that the signal appears as long as the strip is functioning properly even if there is no target analyte. The presence of the control line's signal means that the strip operated properly, and its absence means the opposite. The open source antibody dispenser is capable of dispensing two reagents by attaching two antibody spindles, demonstrating its expandability (Fig. 17).

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Declaration of interest

Declarations of interest: none.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ohx.2021.e00188>.

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