Identification for a Large-volume Food-borne Bacteria on a Fully Integrated Portable Centrifugal Disc

Hau Van Nguyen, Van Dan Nguyen, Eun Yeol Lee and Tae Seok Seo
Department of Chemical Engineering, Kyung Hee University, Yongin 17104, South Korea

Keywords: Centrifugal Microdevice, Portable Genetic Analyser, Loop-mediated Isothermal Amplification, Colorimetric Detection, Super Absorbent Polymer.

Abstract: Herein, we present a fully integrated portable centrifugal microsystem for multiplex detection of food poisoning bacteria with a large volume of sample up to 1 mL. The microsystem consists of a portable rotary genetic analyzer and a fully integrated lab-on-a-disc device. The portable rotary genetic analyzer is equipped with a couple of heating blocks, a motor and a UV-Vis optical detector. The device was designed with two units: a 3D printed solution-loading cartridge and a centrifugal microfluidic disc. All the essential solutions for the LAMP reaction (a sample, a washing, an elution and a LAMP cocktail solution) are stored inside the cartridge, and orderly released into centrifugal microdevice by a rotation program. Each unit of the device was designed with 20 reaction chambers for simultaneously detecting 19 kinds of food poisoning bacteria in one test. To increase the amount of a sample to 1 mL, we incorporated the super absorbent polymer (SAP) in the waste chamber to absorb the sample and washing solution during the device operation. The whole process was automatically conducted from bead-based DNA extraction to isothermal DNA amplification by EBT-mediated LAMP reaction to colorimetric and UV-vis detection of amplicons in 60 min to identify three kinds of bacteria (Escherichia coli O157:H7, Salmonella Typhimurium, and Vibrio parahaemolyticus).

1 INTRODUCTION

Point-of-care testing (POCT) is recently blooming up and plays a vital role in supporting immediate treatment. The central laboratories are equipped with high cost and automatic diagnostic platform for highly sensitive, precise and accurate testing. However, due to the bulkiness of the analytical instruments, they are not adequate for on-site diagnostics. On the contrary, the POC testing allows simple and rapid analysis, which can help the doctor to make a timely decision on the treatment method for the patients. Also, the POC testing offers a user-friendly prototype suitable for the un-trained worker, minimal operation steps to reduce analysis time, minimizing sample storage and transportation, and cost-effective treatment in resource-limited environments. The POC testing system includes a paper-based microfluidic device (PPM) (Choi, 2015; Ye, 2018), a lateral flow assay (LFA) (Deng, 2018; Takalkar, 2017), a microfluidic device (DuVall, 2017; Zhang, 2017), a miniaturized PCR platform (Guarnaccia, 2017; Liu, 2017), a smartphone-based device (Berg, 2015; Priye, 2016; Stedtfeld, 2012; Wang, 2017). Among these POCT approaches, a microfluidic device has attracted huge attention over decades, and, in particular, a centrifugal microfluidic was considered as a promising candidate for complex diagnostic purposes. Centrifugal microfluidics have demonstrated the high fidelity for the unit operation and integration on a single device such as sample loading and reagent storage (Stumpf, 2016; van Oordt, 2013), serial dilution (Kim, 2018), metering, aliquoting, mixing, and incubation (Jung, 2015; Oh, 2016; Park, 2017), and detection (Andreasen, 2015; Martin, 2017; Schwemmer, 2016). However, the major challenge in the centrifugal microdevice is the low volume of sample pretreatment, which could affect the limit-of-detection level. In this study, we proposed a prototype of a fully integrated centrifugal device for the POC testing, which is capable of multiplex bacteria detection with a large volume of the sample up to 1mL. developed for multiplex bacteria detection in a large volume of the sample up to 1mL. In addition, this sample-to-answer platform can fulfill all the requirements for on-site nucleic acid analysis since the DNA solid-phase extraction by glass bead, isothermal amplification by LAMP

Van Nguyen, H., Nguyen, V., Lee, E. and Seo, T.
Identification for a Large-volume Food-borne Bacteria on a Fully Integrated Portable Centrifugal Disc.
DOI: 10.5220/0007690401140118
In *Proceedings of the 12th International Joint Conference on Biomedical Engineering Systems and Technologies (BIOSTEC 2019)*, pages 114-118
ISBN: 978-989-758-353-7

reaction, and amplicon quantification by a UV-Vis detector can be performed on the integrated centrifugal microdevice in a portable genetic analyser system.

2 EXPERIMENTAL

2.1 Design of the Centrifugal Device

The disc was designed to perform the DNA solid-phase extraction and high-throughput LAMP assay with 20 reaction chambers. We proposed the device with two units: a centrifugal microdevice for DNA extraction and LAMP reaction, and a 3D-printed cartridge for solution loading. The centrifugal microdevice was designed with AutoCAD, and etched in a 3.0 mm thick poly(methylmethacrylate) (PMMA) plate using a CNC machine. All the siphon channels were coated with a hydrophobic reagent, Vistex 111-50. The positive reaction chamber was coated with a primer set of the target bacteria. The waste chamber was integrated with super absorption polymer (SAP) from baby diaper for utterly absorbing 1 mL sample solution. A pressure sensitive adhesive (PSA) foil layer was applied to seal the disc. The acid wash glass bead (150-212 µm, Sigma) was then packed into the DNA extraction channel. Finally, the bead-packed channel was incubated in 6M Gu-HCl for 30 min to enhance the DNA capture capacity.

2.2 Portable Rotary Platform with UV-Vis Detector

To adapt our system for POC testing, we also proposed a portable compact and small size rotary platform for operating the disc. The rotary platform consists of: (1) a spindle motor, (2) a couple of Minco heater and (3) a UV-Vis optical detector. The UV-Vis optical system is composed of a yellow LED 570 nm and a red LED 650 nm directed toward LAMP reaction chambers through an optical fiber. A filter was used for eliminating the interference from excited light and an aspheric lens for reducing optical aberrations. The transmittance light intensity was then measured by a CMOS camera sensor and converted into relative absorbance.

2.3 Procedure for the on-Chip Genetic Analysis

Firstly, 1 mL of sample lysis mixture was prepared containing 500 µL of bacteria sample, 250 µL of AL buffer (Qiagen, Netherlands), and 250 µL of 6 mM Gu-HCl (Thermo Fisher Scientific, USA). Sample lysis mixture, a washing solution (70% Ethanol), an elution solution (DNase/RNase water), and a LAMP/EBT cocktail solution were then injected into the cartridge at the injection hole. An in-house program automatically performed all the operation steps including spinning for solution transferring,

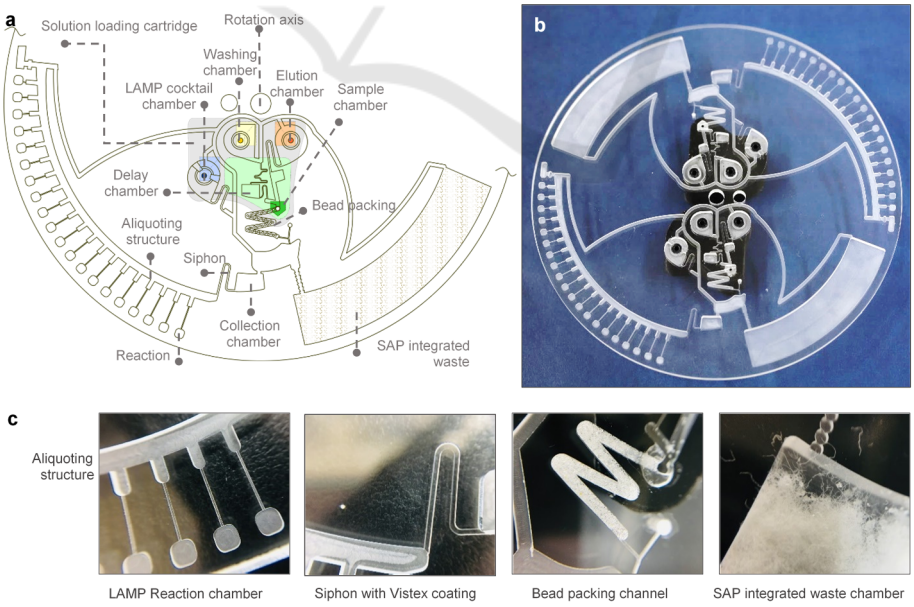

Figure 1: (a) Schematic illustration of the integrated centrifugal microdevice. (b) Digital images of the disc. (c) Components of the microdevice, (i) Aliquoting structure and LAMP reaction chamber, (ii) Siphon channel coated with Vistex, (iii) Glassbead-packed channel for DNA extraction, and (iv) Super Absorption Polymer (SAP) integrated waste chamber.

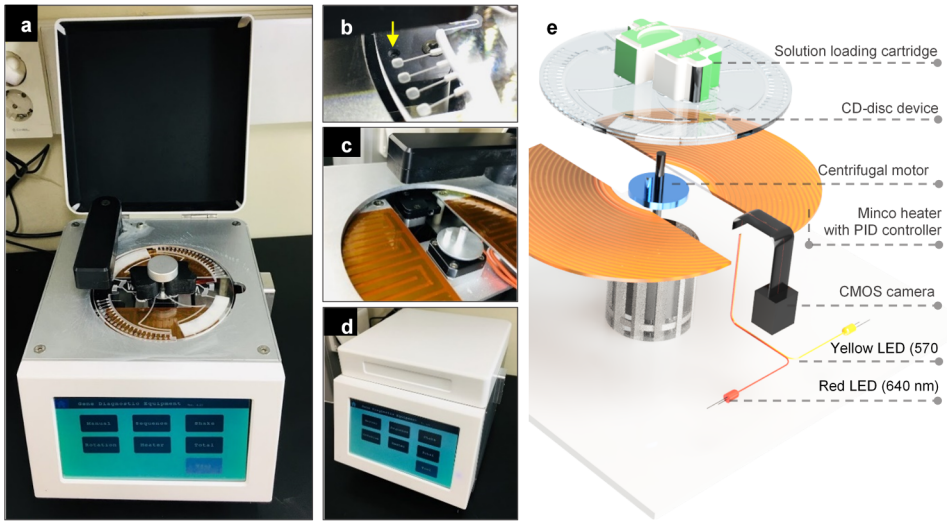

Figure 2: (a) Digital images of the portable rotary platform. (b) UV-Vis detector for measuring absorbance of reaction chamber. (c) A couple of Minco heater. (d) Digital images of the portable rotary platform with closed lib. (e) Schematic illustration of the rotary platform with a centrifugal motor, a couple of Minco heater, and a UV-Vis detector with two light emitting diode (LED) light source at 640 and 570 nm.

shaking for mixing, heating for proceeding LAMP reaction, measuring solution absorbance in real-time, and data production. The absorbance at 640 nm (Abs640) and 570 nm (Abs570) were recorded at a 5 min interval time during 60 min of LAMP reaction. The ratio of Abs640 to Abs570 (Abs640/Abs570) was then calculated. The real-time curve was plotted between Abs640/Abs570 ratio and time.

3 RESULTS AND DISCUSSION

3.1 Optical Real-Time Sensing LAMP Reaction Amplicon by in-House Building System

We recorded the UV-Vis absorption spectrum of the LAMP mixture before and after LAMP reaction. The color of LAMP mixture changed from violet to sky blue during the process of a LAMP reaction with the change of maximum absorption wavelength from 570 nm to 640 nm, respectively. Therefore, we designed the UV-Vis detector on the portable rotary platform with the two LED light source at 570 and 640 nm. We measured the relative absorbance at 640 nm and 570 nm of negative (NC) and positive (PC) chamber with 5 min interval time during the LAMP reaction. The NC chamber has no change in the Abs640/Abs570 ratio. In contrast, for the PC chamber, the Abs640/Abs570 ratio has a change when the LAMP reaction occurs at 40-50 min and became saturated at 55-60 min. These results are in a good agree-ment with UV-vis absorption spectrum of the NC and PC. Therefore, the Abs640/Abs570 ratio could be used as the criteria to identify a positive result of which Abs640/Abs570 ratio is higher than 1.0.

3.2 Singleplex and Multiplex Detection on the Integrated Portable System

The disc was designed for processing parallel 2 samples in one run and in 20 reaction chambers for each sample. Theoretically, up to 20 kinds of foodborne pathogens can be simultaneously detected. In this experiment, we targeted three kinds of bacteria (E. coli O157:H7, S. Typhimurium and V. parahaemolyticus) as a model. While no color change was observed in negative control chambers (chambers from left 1,5,9,13,17), the rest chambers with the coated primer for targeting E. coli O157:H7, S. Typhimurium and V. parahaemolyticus exhibited color change from purple into sky blue.

The absorbance of 20 chambers was also measured during the LAMP reaction. The Abs640/570 ratio after the LAMP reaction shows that all the PC chambers with sky blue color had an Abs640/570 ratio higher than 1.0, and all the NC chamber with violet color had an Abs640/570 ratio lower than 1.0. Consequently, we demonstrated that the proposed microdevice could simultaneously detect multiple pathogen targets in 20 reaction chambers.

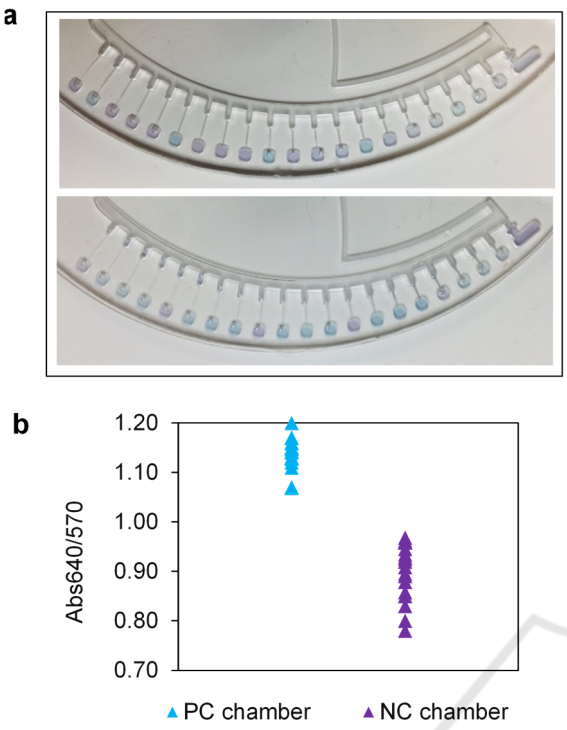

Figure 3: Multiplex detection of foodborne pathogens in samples based on the prototype device. (a) Colorimetric detection of single pathogen (E. coli O157:H7). (b) Colorimetric detection of triple pathogens (E. coli O157:H7, S. Typhimurium and V. parahaemolyticus). (c) The graph of Abs640/570 ratio of negative and positive chamber.

4 CONCLUSIONS

We have developed a sample-to-answer disc for multiplex food poisoning bacteria screening with a large volume of sample (up to 1 mL). The system was automatic and small suitable for POC testing. The disc was designed with the solution-loading cartridges to accomplish a full automation, and a specific SAP integrated waste chamber for large sample volume handling. All experimental processes of the molecular diagnostics were integrated in a single device including extraction, amplification, detection, and data analyzing/reporting. The sample and other essential solutions for LAMP assay are loaded into the 3D printed cartridge, and orderly released into the centrifugal microdevice by a specific channel design and spinning program. The portable genetic analyzer provides a user-friendly interface and a simple operation protocol which is affordable for less technical training staff.

ACKNOWLEDGEMENTS

This work was supported by the Engineering Research Center of Excellence Program of Korea Ministry of Science, ICT & Future Planning (MSIP)/National Research Foundation of Korea (NRF) (2014R1A5A1009799) and by a grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (grant no. HI13C1232).

REFERENCES

Andreasen, S. Z., Kwasny, D., Amato, L., Brøgger, A. L., Bosco, F. G., Andersen, K. B., Svendsen, W. E., Boisen, A., 2015. Integrating electrochemical detection with centrifugal microfluidics for real-time and fully automated sample testing. *RSC Advances 5(22), 17187-17193.*

Berg, B., Cortazar, B., Tseng, D., Ozkan, H., Feng, S., Wei, Q., Chan, R.Y.-L., Burbano, J., Farooqui, Q., Lewinski, M., Di Carlo, D., Garner, O.B., Ozcan, A., 2015. Cellphone-Based Hand-Held Microplate Reader for Point-of-Care Testing of Enzyme-Linked Immunosorbent Assays. *ACS Nano 9(8), 7857-7866.*

Choi, J. R., Tang, R., Wang, S., Wan Abas, W. A. B., Pingguan-Murphy, B., Xu, F., 2015. Paper-based sample-to-answer molecular diagnostic platform for point-of-care diagnostics. *Biosensors and Bioelectronics 74, 427-439.*

Deng, X., Wang, C., Gao, Y., Li, J., Wen, W., Zhang, X., Wang, S., 2018. Applying strand displacement amplification to quantum dots-based fluorescent lateral flow assay strips for HIV-DNA detection. *Biosensors and Bioelectronics 105, 211-217.*

DuVall, J. A., Le Roux, D., Thompson, B. L., Birch, C., Nelson, D. A., Li, J., Mills, D. L., Tsuei, A.-c., Ensenberger, M. G., Sprecher, C., Storts, D. R., Root, B. E., Landers, J. P., 2017. Rapid multiplex DNA amplification on an inexpensive microdevice for human identification via short tandem repeat analysis. *Analytica Chimica Acta 980, 41-49.*

Guarnaccia, M., Iemmolo, R., Petralia, S., Conoci, S., Cavallaro, S., 2017. Miniaturized Real-Time PCR on a Q3 System for Rapid KRAS Genotyping. *Sensors (Basel, Switzerland) 17(4), 831.*

Jung, J. H., Park, B. H., Oh, S. J., Choi, G., Seo, T. S., 2015. Integrated centrifugal reverse transcriptase loop-mediated isothermal amplification microdevice for influenza A virus detection. *Biosensors and Bioelectronics 68, 218-224.*

Kim, T.-H., Kim, C.-J., Kim, Y., Cho, Y.-K., 2018. Centrifugal microfluidic system for a fully automated N-fold serial dilution. *Sensors and Actuators B: Chemical 256, 310-317.*

Liu, W., Zhang, M., Liu, X., Sharma, A., Ding, X., 2017. A Point-of-Need infrared mediated PCR platform with

compatible lateral flow strip for HPV detection. *Biosensors and Bioelectronics 96, 213-219*.

Martin, J. W., Nieuwoudt, M. K., Vargas, M. J. T., Bodley, O.L.C., Yohendiran, T. S., Oosterbeek, R. N., Williams, D. E., Cather Simpson, M., 2017. Raman on a disc: high-quality Raman spectroscopy in an open channel on a centrifugal microfluidic disc. *Analyst 142(10), 1682-1688*.

Oh, S. J., Park, B. H., Choi, G., Seo, J. H., Jung, J. H., Choi, J. S., Kim, D. H., Seo, T. S., 2016. Fully automated and colorimetric foodborne pathogen detection on an integrated centrifugal microfluidic device. *Lab on a Chip 16(10), 1917-1926*.

Park, B. H., Oh, S. J., Jung, J. H., Choi, G., Seo, J. H., Kim, D. H., Lee, E.Y., Seo, T. S., 2017. An integrated rotary microfluidic system with DNA extraction, loop-mediated isothermal amplification, and lateral flow strip based detection for point-of-care pathogen diagnostics. *Biosensors and Bioelectronics 91, 334-340*.

Priye, A., Wong, S., Bi, Y., Carpio, M., Chang, J., Coen, M., Cope, D., Harris, J., Johnson, J., Keller, A., Lim, R., Lu, S., Millard, A., Pangelinan, A., Patel, N., Smith, L., Chan, K., Ugaz, V.M., 2016. Lab-on-a-Drone: Toward Pinpoint Deployment of Smartphone-Enabled Nucleic Acid-Based Diagnostics for Mobile Health Care. *Analytical Chemistry 88(9), 4651-4660*.

Schwemmer, F., Blanchet, C.E., Spilotros, A., Kosse, D., Zehnle, S., Mertens, H.D.T., Graewert, M.A., Rössle, M., Paust, N., Svergun, D.I., von Stetten, F., Zengerle, R., Mark, D., 2016. LabDisk for SAXS: a centrifugal microfluidic sample preparation platform for small-angle X-ray scattering. *Lab on a Chip 16(7), 1161-1170*.

Stedtfeld, R. D., Tourlousse, D. M., Seyrig, G., Stedtfeld, T. M., Kronlein, M., Price, S., Ahmad, F., Gulari, E., Tiedje, J. M., Hashsham, S. A., 2012. Gene-Z: a device for point of care genetic testing using a smartphone. *Lab on a Chip 12(8), 1454-1462*.

Stumpf, F., Schwemmer, F., Hutzenlaub, T., Baumann, D., Strohmeier, O., Dingemanns, G., Simons, G., Sager, C., Plobner, L., von Stetten, F., Zengerle, R., Mark, D., 2016. LabDisk with complete reagent prestorage for sample-to-answer nucleic acid based detection of respiratory pathogens verified with influenza A H3N2 virus. *Lab on a Chip 16(1), 199-207*.

Takalkar, S., Baryeh, K., Liu, G., 2017. Fluorescent carbon nanoparticle-based lateral flow biosensor for ultrasensitive detection of DNA. *Biosensors and Bioelectronics 98, 147-154*.

Wang, K., Liang, R., Chen, H., Lu, S., Jia, S., Wang, W., 2017. A microfluidic immunoassay system on a centrifugal platform. *Sensors and Actuators B: Chemical 251, 242-249*.

Zhang, L., Ding, B., Chen, Q., Feng, Q., Lin, L., Sun, J., 2017. Point-of-care-testing of nucleic acids by microfluidics. *TrAC Trends in Analytical Chemistry 94, 106-116*.