POINT-OF-CARE DIAGNOSIS OF INFECTIOUS DISEASES BY VARIOUS LABORATORY DEVICES AND SYSTEMS

Emilia Georgieva
TS Medical Laboratory Assistant, Medical College, Medical University of Varna, Bulgaria
Emiliya.Georgieva@mu-varna.bg
Yordanka Mihaylova
TS Medical Laboratory Assistant, Medical College, Medical University of Varna, Bulgaria
jordanka.mihaylova@mu-varna.bg

Abstract: We live in an unusual epidemic situation that no one has ever anticipated. The COVID-19 pandemic has shown us how vulnerable we are when it comes to different disasters and has created serious challenges for the healthcare systems. The early detection, early isolation, and early treatment, which will limit the spread of the virus in the population, are directly related to the diagnostic methods, especially the quick, accessible, and inexpensive ones, as well as those near the patient (point-of-care diagnostics). Instead of the usual methods of detection and confirmation of viruses used in laboratory diagnostics, various modified polymerase chain reaction (PCR) devices are being introduced. The focus of this article is microfluidics in combination with the microelectromechanical system technology, which allows miniaturization of PCR processes using a lab-on-a-chip device with the potential advantages being ultrafast speed, low cost and low sample volumes, portability, high output, and integration and automation options.
Keywords: point-of-care diagnostics, infectious diseases, COVID-19, qPCR

1. INTRODUCTION

Point-of-care nucleic acid tests (POC NATs) are based on the nucleic acid amplification technique (NAT) and their aim is realization of quick and simple automatic detection. Due to the progress in manufacturing, digital, and artificial intelligence technologies, and bio-informatics in the recent years, the development of the POC NAT device has led to a significant advance. Instead of the regular methods for nucleic acid detection used in laboratory diagnostics, some new experimental carriers have been used. The implementation of experimental carriers has contributed to the automation and integration of nucleic acid detection by several steps (nucleic acid extraction, amplification and detection of the signal). The manufacture of these technologies is complex. However, they are accurate, ensure a significant advance in nucleic acid detection, and accomplish the integration in nucleic acid detection. In Bulgaria, even before the pandemic, we had experience with working on some devices for quicker polymerase chain reaction (PCR) diagnostics, like GeneXpert, which were regularly used during the COVID-19 pandemic. One disadvantage of the method is its cost, which is still high in our country.

AIM: The aim of the article is to present the trends in infectious disease diagnostics by new laboratory methods and miniature PCR devices.

MATERIALS AND METHODS: We have used a documentary method and consulted 18 scientific publications on the research topic from European and other countries.

2. RESULTS

- **Miniature PCR Devices**

Polymerase chain reaction is among the most popular detection methods and a gold standard in the molecular diagnostics of infectious diseases. It allows exponential amplification of one or multiple copies of a specific DNA or RNA sequence, which makes it highly potent, specific, and sensitive (4;8;11). The typical PCR processes use a small volume of no more than 100 μL and the reaction takes place at cyclic temperatures between 60°C, 72°C, and 95°C. Despite this, the conventional PCR devices are generally complex, large, and expensive. They are used only in diagnostic laboratories, which excludes their use in home settings near the patients. The emergence of microfluidics in combination with the microelectromechanical system technology has allowed the miniaturization of the PCR processes using a lab-on-a-chip device with the potential advantages being ultrafast speed, low cost and low sample volumes, portability, high output, and integration and automation options (7;17). This will allow the application of PCR-based diagnostics in resource-poor regions. Despite this, PCR processes integrate precise heating and cooling management modules for thermal cycles, and increase the challenges and expenditure for miniaturization, which requires a more complex and detailed design for optimization of the thermal properties (3;16;18). The emergence of quantitative PCR (qPCR) has contributed to nucleic acid amplification and quantitative

real-time detection, all in one step, without electrophoresis or hybridization stages, which is the pioneer of PCR-based integrated NAT, despite its complex and precise heating and cooling control (1;5;11). In order to achieve quick or ultrafast NAT, the simplification and shortening of the conventional PCR processes are the two key challenges to which attention should be paid, because the heating/cooling speed of conventional PCR systems is only a few degrees Celsius per second and a long time, 1 to 2 hours, is needed for the completion of the entire PCR process. This indicates the need of improvement in order to have a quick diagnostic process. In newly introduced solutions, including increase of the speed of thermal conductivity, the most frequently used approaches are efficient heaters and a decrease in the sample volume (9;12;15). The low sample volume is the best option due to its cost-effectiveness and easy change in the speed of heating or cooling when microfluidics is used.

- **Virus Detection**

A team of authors has developed a microfluidic chip system for sensitive SARS coronavirus detection (15). This system consists of a laser-induced fluorescent microfluidic chip analyzer, a glass microchip both for PCR and for capillary electrophoresis, chip thermal cycling based on double thermoelectric elements, RT-PCR diagnostic kit for SARS and a sizing kit for DNA electrophoresis. This system allows efficient amplification of the SARS-CoV cDNA with a subsequent electrophoresis on the same glass chip. Then the results from the electrophoresis on the microchip are collected and analyzed by laser-induced fluorescent microfluidic chip analyzer. The results from the test of this system show that 17 positive probes are received out of 18 nasopharyngeal tampon probes from clinically diagnosed patients, which demonstrates high positive frequency, precision, and speed of the microfluidic chip system for SARS diagnostics (6;10;13).

In addition, due to the significant advantage of qPCR and its gold standard for molecular detection, the miniaturization of the qPCR devices will contribute considerably to the development of POC tests for infectious diseases. The developed POC device, based on qPCR, combined with nucleic acid extraction with magnetic nanoparticles for automated adenovirus detection (2;9;18) would contribute significantly to the diagnostic process.

3. CONCLUSIONS

This review article presents the trends and the progress in the development of POC NAT devices, which allow the integration of a multistage NAT on specific carriers. The time until obtaining the results is very short, thus simplifying the traditional NAT. The POC NAT devices are moving away from the diagnostic laboratories; they are easy to use in field conditions or near the patient. This is a revolutionary change for NAT and has wide application perspectives.

The current field of diagnostics should focus on a one-way analysis since this system type is easier to use, easier to analyze, and more suitable for mass production. The implementation of multiplex devices can improve the accuracy, sensitivity, and scalability of the research and diagnostic devices applicable in field conditions and especially suitable for resource-poor regions. The development of POC NAT devices for first aid will increase considerably the potential of the diagnostic process. Further improvements of the NAT and POC technology will lead to significant expansion of their possible applications.

REFERENCES

Broeders, S., Huber, I., Grohmann, L., Berben, G., Taverniers, I., Mazzara, M., Roosens, N., & Morisset, D. (2014). Guidelines for validation of qualitative real-time PCR methods, Trends Food Sci. Technol. 37.115–126.

Chen, H., Wu, Q., Chen, Z., Hu, L., Fang, L., Liao P., Deng Y., & He, Y. (2017). Performance evaluation of a novel sample in–answer out (SIAO) system based on magnetic nanoparticles, J. Biomed. Nanotechnol. 13. 1619–1630.

Dai, F., Furst A., & Liu, C. (2019). Strand displacement strategies for biosensor applications, Trends Biotechnol. 37. 1367–1382.

Furst, L., & Francis, B. (2019). Impedance-based detection of bacteria, Chem. Rev. 119. 700–726..

Neuzil, P., Zhang, Y., Pipper, Oh, S., & Zhuo, L. (2006). Ultra fast miniaturized real-time PCR: 40 cycles in less than six minutes, Nucleic Acids Res. 34. e77.

Nzelu, O., Kato H., & Peters, C. (2019). Loop-mediated isothermal amplification (LAMP): an advanced molecular point-of-care technique for the detection of Leishmania infection, PLoS Negl. Trop. Dis. 13. e0007698.

Park, S., Zhang, Y., Lin, S., Wang, H., & Yang, S. (2011). Advances in microfluidic PCR for point-of-care infectious disease diagnostics, Biotechnol. Adv. 29. 830–839.

Postollec, F., Falentin, H., Pavan, S., Combrisson, J., & Sohier, D. (2011). Recent advances in quantitative PCR (qPCR) applications in food microbiology, Food Microbiol. 28. 848–861.

Quyen, L., Ngo, A., Bang, D., Madsen, M., & Wolff, A. (2019). Classification of multiple DNA dyes based on inhibition effects on real-time loop-mediated isothermal amplification: prospect for point of care setting, Front. Microbiol. 10. 2234.

Salman, A., Carney, H., Bateson, S., & Ali, Z. (2020). Shunting microfluidic PCR device for rapid bacterial detection, Talanta. 207. 120303.

Toze, S. (1999). PCR and the detection of microbial pathogens in water and wastewater, Water Res. 33. 3545–3556.

Ullerich, L., Campbell, S., Krieg-Schneider, F., Bursgens, F., & Stehr, J. (2017). Ultra-fast PCR technologies for point-of-care testing, LaboratoriumsMedizin. 41. 239–244.

Wan, L., Gao, J., Chen, L., Dong, C., Li, R., Wen, Z., Lun, R., Jia, W., Mak, I., & Martins, P. (2019). LampPort: a handheld digital microfluidic device for loop-mediated isothermal amplification (LAMP), Biomed. Microdevices. 21. 9.

Yeo, J., Kang, H., Dao, D., Cuc, T., Nguyen, V., Tien, T., Hang, K., Phuong, M., Thanh, T., Mai, Q., Rah, Y., Yu, K., Shin, J., Chong, K., Choi, S., & Park H. (2018). Development of a smartphone-based rapid dual fluorescent diagnostic system for the simultaneous detection of influenza A and H5 subtype in avian influenza A-infected patients, Theranostics 8. 6132–6148.

You, L., Cao, L., & Xu, F. (2020). Plasmon-driven ultrafast photonic PCR, Trends Biochem. Sci. 45. 174–175.

Zhang, S., & Xing, D. (2007). Miniaturized PCR chips for nucleic acid amplification and analysis: latest advances and future trends, Nucleic Acids Res. 35. 4223–4237.

Zhang, S., Xu, L., Ma, L., & Zheng, L. (2006). PCR microfluidic devices for DNA amplification, Biotechnol. Adv. 24. 243–284.

Zhang, H., Xu, Y., Fohlerova, Z., Chang, H., Iliescu, C., & Neuzil, P. (2019). LAMP-on-a-chip: revising microfluidic platforms for loop-mediated DNA amplification, TrAC Trends Anal. Chem. 113. 44–53.