

Advancement in POCT molecular testing: the multiplex PCR POCT devices for infectious diseases

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ABSTRACT

Rapid and accurate diagnostic tests are very important for the global control of infectious diseases. The point of care diagnosis has become a promising strategy in recent years. Different kind of point of care testing devices has been introduced into the market in the last decade. These devices must provide a low-cost, robust, sensitive, specific, and practical analysis in order to replace the conventional clinical laboratory diagnostic test algorithms when needed. The successful implementation of point of care diagnostics has a potential to increase the strength of infectious diseases surveillance programs. Finally, the rapid progress in point of care diagnosis can stimulate a shift from a centralized diagnostic model to a decentralized patient-centered approach.

INTRODUCTION

It is critically important to reduce the global burden of infectious diseases and the drug resistant cases. Because of this reason, new diagnostic methods and instruments are continuously being developed through basic research. Rapid diagnosis of infections is very important for the initiation of an effective treatment.

After the discovery of polymerase chain reaction (PCR), there are many important milestones including sequencing, microchip technology, real-time PCR, and cartridge tests in the history of diagnostic molecular tests. Actually this amazing history is not more than 40 years that is a tiny space in the history of human being. The first step of technological progress is dreaming as stated in a paper published in 2002 by Dunne et al (1).

In that paper the authors introduced a mythical take on the future of the clinical microbiology laboratory based on the technological progress and describe the clinical microbiology in the year 2025. One of their most important predictions was the reversion of the trend of centralized laboratory services towards a decentralized testing approach that was basically point of care testing. Based upon the evolutionary pace of the technology, it will not be surprising to see that most of their predictions will become true until that time.

MULTIPLEX POINT OF CARE TECHNOLOGIES

Multiplex point-of-care diagnostic technologies (MPOCTs) can test the presence of multiple infectious pathogens within a specimen (such as blood, urine, or sputum) (2). Proteins, cells, DNA, RNA, exosomes and metabolites can be detected by using MPOCT devices that contain hybridization papers, array settings, bead technology or microfluidic systems. This theoretically complex high technology design actually provides a very practical application that can be performed in

places outside the routine laboratories like clinics, wards or doctor's offices. The favorable outcome for an ideal MPOCT device is to have a high sensor performance at low system complexity. The test results can be obtained within 15 minutes to several hours. The development of new molecular panel diagnostics that can provide results in 15 minutes would provide both clinical and economic benefits. Analysis of the multiplexed results provides the clinician with an opportunity to administer personalized therapies in a short time.

Potential benefits of MPOCTs for infectious diseases include improved patient health care and management, more appropriate use of antibiotics, improved ability to limit the spread of disease, health care cost savings and increased access to testing in remote or low-resource settings (3).

The set of tests on a multiplex technology is known as a test panel. Syndromic test panels are designed to test for multiple diseases associated with a similar set of symptoms, or a syndrome. These panels help the evaluation of the cause of the disease at the point of care. Respiratory panels and gastrointestinal panels are two examples of syndromic panels (4,5).

The main performance characteristics of commercially available MPOCTs (6) include:

1. panel size, or the number of pathogen targets that can be tested in one sample run;
2. time to test result;
3. throughput, or the number of patient samples that can be run simultaneously; and
4. physical size of the device.

The molecular diagnostic methods were initially expensive due to high investment costs, had long turnaround time (hours), and needed experienced user. However, recent developments in isothermal DNA amplification have made great contribution to the workflow of molecular diagnostics (7,8).

The successful migration of high sensitivity molecular diagnostics from the routine diagnostic laboratories to the field could dramatically improve the accuracy and sensitivity of MPOCTs, enhance public health reporting, and facilitate outbreak containment in difficult settings (9).

The key design features for MPOCTs in resource-limited settings included: loop-mediated isothermal amplification to eliminate the need for a thermal cycler, lyophilized reagents for long-term stability at high temperature, and relatively simple procedures for ease-of-use by operators in a field laboratory (10).

Microfluidic devices can provide a fully integrated MPOCT device for sample processing, fluid handling, and signal generation (11,12). A major goal is a low-cost diagnostic test for use in remote settings. Microfluidics-based devices use channels to transport small amounts of fluid by actuation forces. Solid phase nucleic acid extraction and isothermal enzymatic nucleic acid amplification steps are combined on microfluidic cartridges or chips that contain pre-stored, paraffin-encapsulated lyophilized reagents (13).

The use of microfluidic technologies reduces assay complexity and enables multiplex analysis and high-throughput screening (14). On-chip nucleic acid analysis is particularly promising because it miniaturizes and integrates the various assay steps, including the lysis or extraction of target cells, the purification of nucleic acids, the amplification of nucleic acids, and on-chip detection of reaction products (15). Current efforts in the development of lab-on-chip diagnostics include the identification of new biomarkers, as well as integrated microfluidic design, construction materials, and detector technologies (16).

A particular concern is the per-test cost and the need for instrumentation to drive the devices and product detection. One novel approach to assay construction is the use of layered paper to construct three-dimensional microfluidic

devices that can distribute fluids vertically and horizontally and enable streams of fluid to cross one another without mixing (17).

With regard to detector technologies, a universal mobile electrochemical detector was recently described that can communicate results to distant sites using a mobile phone (13). These and similar developments will be critical for lab-on-chip diagnostics for resource-limited settings (18,19).

Effective communication of results for disease surveillance can be best accomplished if there is standardization for result recording and reporting. Ideally, multiple detection technologies might be combined in a single instrument.

The communication of results from MPOCT assays will require the abilities to digitally capture data and to communicate results to a central database. But using an electronic reader to scan point of care tests and store or transmit patient data remain as a major concern for data privacy and security.

The immediate goal of a MPOCT assay is to use the information gained from the test to impact the care of the patient. For many diseases, particularly, communicable diseases such as influenza or emerging infectious diseases, the use of MPOCT assays can provide a key element of disease surveillance (9). Linking data to specific geographical locations can provide information regarding disease emergence, disease spread, or progress toward control.

There are some studies that evaluate the effectiveness of MPOCTs compared to routine, laboratory-based detection methods in order to assess the impact on length of stay and antibiotic usage. In one of these studies, for the first time, the ward staff performed the MPOCT (20). The authors found no association between respiratory PCR MPOCT testing and length of stay or most of the secondary outcomes except the antimicrobial prescribing decision. They concluded

that this was probably due to a delay in initiating MPOC testing. MPOC testing allowed time-critical antivirals to be given significantly faster, and results were available considerably faster than routine laboratory-based testing. It seems that in order to obtain the most beneficial results from MPOCTs, the tests must be performed immediately after the collection of the specimens. This action requires at least one personnel to be allocated for the application of MPOCTs. As highlighted by the authors, new technology itself is not enough, it should be incorporated into the routine algorithms in a correct manner.

CONCLUSION

An ideal device for multiplexed point-of-care testing should offer a high sensor performance, like high sensitivity and multiplexing capability, as well as short turnaround times, at low system complexity, including low-cost fabrication and minimized user intervention. The future technology challenges will be the standardization and further miniaturization of the system components for the most effective use of this tool as a part of infectious diseases surveillance programs. It seems that the more widely use of MPOCT devices will bring diagnostic testing closer to patients and will be a driving force for a shift from a centralized model to a decentralized patient-centered approach.

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