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REVIEW ARTICLE

From qPCR to Next-Generation Sequencing: Practical Strategies for Resource-Limited Molecular Laboratories

Imène BENAMIRA[1,4], Zouhir DJENNA[2,5], Louisa BECHOHRA[3]

ABSTRACT

Personalized medicine, also referred to as precision medicine, has been enabled by emerging technologies such as next-generation sequencing (NGS), particularly through applications like liquid biopsy, where genetics and genomics play a central role. Consequently, the integration of NGS into clinical and research settings has become increasingly essential. However, incorporating NGS into molecular biology platforms traditionally based on quantitative polymerase chain reaction (qPCR) represents a significant challenge in Algeria and other resource-limited settings. This transition requires substantial adaptations in laboratory infrastructure and workflow design, as well as the implementation of robust quality management systems. Additional challenges include personnel training, assay validation, data management, regulatory compliance, cost sustainability, and workforce development. This literature review aims to identify and analyze potential solutions to the challenges associated with implementing NGS within qPCR-based molecular biology platforms in resource-limited countries, with a particular focus on Algeria.

Keywords: Personalized medicine, next-generation sequencing (NGS), quantitative PCR (qPCR), liquid biopsy, resource-limited countries, installation of NGS, quality management systems, personnel training.

1- Department of Pathology, Hussein-Dey Hospital, Algiers, Algeria. 2- Department of Neurosurgery, Benflis Touhami Hospital, Batna, Algeria. 3- Faculty of Biological Sciences, University of Science and Technology Houari Boumediene (USTHB), Algiers, Algeria. 4- Faculty of Medicine, University of Health Sciences, Algiers, Algeria. 5- Faculty of Medicine, Batna, Algeria.

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Correspondance to: Imene BENAMIRA
E-mail: benamiraimene2@gmail.com

1. INTRODUCTION

In resource-limited countries, molecular platforms are mostly based on qPCR, providing reliable and cost-effective results, particularly for infectious diseases and cancer. However, the rapid evolution of personalized medicine has highlighted the limitations of molecular tests that aim to detect a single abnormality and the growing preference for multiplex gene analysis. NGS addresses this need by enabling the parallel analysis of multiple target genes, thus contributing to targeted therapy. Nevertheless, many platforms, especially in resource-limited countries, have not yet been able to implement this innovative technology, primarily due to infrastructural constraints, workforce limitations, regulatory challenges, and economic barriers. This article aims to provide a practical approach to introducing NGS into qPCR platforms by summarizing international guidelines and expert recommendations (1-4).

2. CURRENT STATE OF qPCR LABORATORIES IN RESOURCE-LIMITED SETTINGS

In Algeria, most molecular platforms are based on qPCR. The standard differs slightly from international recommendations: the physical separation of pre- and post-amplification areas is often incomplete, and many laboratories have two or three rooms connected by standard doors without a dedicated ventilation system for each zone. Air therefore circulates freely between clean and contaminated areas. Automated extraction systems, dedicated laminar flow workstations, transfer airlocks, and backup power sources (UPS) are not always available. Furthermore, equipment maintenance is often a major challenge in these countries.

However, technicians are well-trained and follow the workflow: they begin with sample preparation, then reagent preparation, and

finally amplification. They also adhere to hygiene measures, such as wearing lab coats, frequently changing gloves, and using UV hoods. The transition to NGS, in this specific context, requires a reorganization of the architecture, biosafety protocols, and workflows in order to meet international standards (5-7). In Algeria, and particularly in the field of pathology, there are few molecular biology platforms. They are also relatively new. No studies have yet been published on this subject. The observations reported here are based on the authors' experience.

3. CHALLENGES IN RESOURCE-LIMITED qPCR LABORATORIES

Although results are generally reliable, several factors can affect quality. These factors include infrastructure, and technical and organizational constraints. Despite the use of UV-equipped laminar flow hoods, the risk of contamination remains significant due to inadequate physical separation and the absence of a dedicated ventilation system for each zone. Other challenges include logistical delays, inconsistent quality management systems, and limited participation in external quality assessment programs due to funding constraints (8). Below are the essential requirements that laboratories should address before and during NGS implementation:

Laboratory Infrastructure and Workflow Design

International experts recommend functional separation in laboratories based on qPCR and would like to add NGS. At least two zones are required: a pre-PCR and a post-PCR. The pre-PCR zone is a clean area where nucleic acid extraction, reagent preparation, and the initial library preparation steps take place. If possible, these different steps are functionally separated with dedicated benches, equipment, and temporal separation. Furthermore, strict measures should be implemented to control contamination, such as maintaining DNA- and RNA-free work surfaces, careful pipetting techniques, rapid sealing of reaction tubes, frequent glove changes, and routine surface decontamination (4,9,10).

Quality Management and Control Systems

To ensure quality management and assurance (QMA), it is essential to begin by documenting the entire process in documents such as standard operating procedures (SOPs). This ensures that the technique is reproducible and can be verified by accreditation bodies such as the American College of Pathologists (CAP).

Furthermore, to ensure the reliability of the technique, each step should undergo quality control (QC). QC parameters include base quality and coverage thresholds. The use of positive and negative controls allows for the detection of contamination and ensures the reliability of the technique (11).

Personnel Training and Expertise

To acquire NGS in a lab with qPCR, staff must undergo NGS training; each technician on the new platform must be versatile and master several steps of the NGS workflow, such as sample handling, library preparation, and bioinformatics analysis. Quality assurance practices must be learned and respected; staff must also learn how to solve technical problems. One of the major problems encountered is the departure of biologists after training, which leads to a loss of skills. To address this, standardized training programs and official competency assessments must be used (12).

Validation and Standardization of Assays

Validation of the technique is mandatory before starting the procedure. This validation is done by determining the sensitivity, specificity, reproducibility, and limit of detection for different samples. Depending on the laboratory's resources, pre-validated commercial panels may be used, or internal tests specific to each laboratory may be performed (4).

4. BEYOND qPCR: NAVIGATING THE COMPLEXITY OF END-TO-END NGS WORKFLOWS

NGS workflow is very complex. It includes a pre-analytical stage (wet lab), an analytical stage corresponding to library preparation and sequencing, and post-analysis, which involves the bioinformatics pipeline. NGS is far more complex than qPCR, using significantly more reagents, hardware, and software. A change in even one element needs a reassessment of the entire system to ensure the final result remains accurate. For laboratories undergoing a transition, viewing NGS as a unified, end-to-end process is vital for maintaining the diagnostic chain of custody and ensuring reproducible results (4,12,13).

Technical Implementation and Platform Selection

First, the sequencer must be chosen, primarily suited to the laboratory's infrastructure and budget. High-throughput platforms (e.g., IlLumina NextSeq, NovaSeq, etc.) are multiplexed and generally used in large centers. The cost per sample is low. However, these

systems are expensive and require many samples per cycle to be cost-effective. Furthermore, they require a sophisticated environment, including an industrial cooling system, a high-capacity stabilizer, and a suitable storage system. Benchtop platforms (e.g., MiSeq, Ion GeneStudio S5, Genexus) are compact systems, primarily used during the transition from qPCR to NGS. Their small size allows them to be used by small laboratories or laboratories designed for qPCR. They offer the advantage of being able to analyze a small number of samples. However, their cost is higher than that of high-throughput platforms (4, 13-15).

Data Infrastructure and Bioinformatics Tools

Unlike qPCR, NGS generates large amounts of data. This requires secure storage, structured management, and robust analysis pipelines. The generated and archived data must be backed up and secured. The creation of a laboratory information management system is therefore essential (4,12,13,16).

Regulatory Compliance and Quality Management Frameworks

Compliance with international quality standards is necessary. CLIA (Clinical Laboratory Improvement Amendments) is a mandatory regulatory standard in the United States. ISO 15189 is an international standard considered a benchmark for the accreditation of medical laboratories. If the laboratory uses FDA (Food and Drug Administration)-approved tests, the laboratory must follow FDA regulations (4,12,17,18).

Cost Planning and Economic Sustainability

The installation of NGS remains expensive despite the decrease in sequencer prices in recent years, primarily due to the cost of consumables, data storage, and staff training. However, current trends highlight the potential of NGS, particularly its financial advantages, in the context of personalized medicine, where it is often necessary to test multiple genes simultaneously. Several studies have reported cost savings with targeted panel sequencing when four or more genes are analyzed (14,19,20). Of course, determining the exact cost of NGS analyses and whether its use truly leads to cost savings requires further research and experimentation.

5. OVERCOMING HUMAN RESOURCE BARRIERS

The biggest challenge is training the staff, who are already well-trained and aware of the risks of contamination. However, the NGS platform requires more knowledge, particularly in quality assurance principles. International experts emphasize the importance of training laboratory staff and assessing their skills. Staff must be empowered with clearly defined tasks. Charters and internal regulations should be in place. Collaboration is essential between pathologists, molecular biologists, and bioinformaticians. This allows for the acquisition of experience and knowledge, reducing the need for expert opinions (4,5,21,22).

6. FUTURE PERSPECTIVES AND STRATEGIC INTEGRATION

The future of NGS lies in liquid biopsy (the analysis of circulating tumor DNA), a non-invasive method that enables real-time tumor profiling, treatment monitoring, detection of resistance mechanisms, and disease surveillance. Liquid biopsy is particularly valuable when tissue sampling is not possible. zNGS should be integrated into national cancer control programs because of its significant potential in personalized medicine (4,23–25).

7. CONCLUSION

Adding NGS to qPCR platforms in resource-limited countries requires the acquisition of new equipment and organizational efforts. This includes strategic planning, functional workflow design, robust quality management systems, and investment in human capital. Integrating NGS platform into molecular biology laboratories is an essential step for the application of personalized medicine. Liquid biopsy is an excellent prospect that allows for rapid implementation of personalized medicine in routine practice.

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