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Multiplex PCR: a powerful and affordable tool for laboratory and field analysis in developing countries

Mohamed O Ahmed*

Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, P.O. Box 13662 Tripoli, Libya

To the editor,

Since the introduction of multiplex-PCR (mPCR) in 1988, this technique has emerged as a highly efficient and sensitive molecular tool for nucleic acid-based diagnosis and monitoring, it is applicable to a broad range of physiological, metabolic and infectious conditions affecting human, animal and plant health[1,2]. Yet, routine use of this molecular technology has not been adopted uniformly among the world's nations, with developed countries being the early adopters and developing countries being slow to exploit this powerful tool[2]. Yet, the high-throughput potential and the promise as a cost-effective assay of mPCR are more suited to the limited resources of developing countries, which are struggling with providing accurate and timely disease management and maintaining adequate and healthy food sources for their populations.

Uniplex PCR is well established and frequently used in clinical and academic settings in developing countries. However, uniplex PCR is performed with a single primer set and can only amplify a single target sequence at once; in contrast, the mPCR method involves multiple primers[2]. The mPCR process is more powerful (capable of amplifying multiple target sequences simultaneously), but it is also more complex and requires careful optimization of conditions and use of appropriate controls in order to ensure the maximum accuracy of results and cost-effective use over time[2]. However, the initial set-up of a mPCR assay may be technically demanding and time-consuming, in the end it provides the benefit of higher throughput and less costly analyses[3-6]. These lastest features are the most beneficial for clinicians/veterinarians, researchers and agricultural regulators in developing countries, but before they can be expected to pursue the use of mPCR over uniplex PCR, a detailed knowledge of the technique and its theory must be provided.

The practical value of mPCR, as a highly sensitive multi-diagnostic method to differentiate genotype and subtype of a broad range of microorganisms, has already been demonstrated for an equally broad range of source materials. Applications in the field of infectious diseases include the identification of pathogenic agents in easily-obtainable specimens from animals (such as blood or fecal matter from humans and domesticated/livestock animals) or the environment (such as plants, soil or water), in order to obtain an initial diagnosis to initiate targeted therapies or to monitor on-going

*Corresponding author: Dr. Mohamed O Ahmed, Assistant Professor, Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, P.O. Box 13662 Tripoli, Libya.
E-mail: a.mo@live.com

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treatment efficacies[3-7]. This type of application is a particularly promising approach for epidemiological surveillence as well[6], for example, mPCR assays can be developed to diagnose and distinguish the established emerging pathogenic infections of particular interest in a particular region, such as pneumonia, tuberculosis and respiratory viral infections[7,8]. A specific advantage of mPCR, compared to the real-time PCR assay, is that it is popular in developed nations, and its lower cost associated with the less costly equipment and reagents; indeed, comparative studies of mPCR and real-time PCR have shown mPCR to be more sensitive and accurate for the detection of bacterial gastrointestinal pathogens (*i.e.* *Helicobacter* spp.) and the analysis of antibacterial drug susceptibility[9].

As mentioned above, a powerful advantage of mPCR is the ability to detect multiple pathogens and various strains in a single sample[10]. An excellent practical example of this is the use of mPCR in developed countries to successfully survey and manage the H1N1 influenza A pandemic in a timely manner[10]. Similar approaches are underway for other more strongly established epidemics, such as HIV/AIDS, hepatitis viruses and respiratory viruses, with the goal of reducing hospitalization rates and costs as well as improving disease outcomes[10,11].

The elegance of all PCR methodologies, including mPCR, is represented by their modifiable nature that allows for continuous improvements in sensitivity and accuracy as well as expansion of the targets within genomic areas[9-11]. Certainly, mPCR can help all developed and developing countries to gain their ultimate goal of improving human health through direct clinical applications, environmental applications or in the food-source industries. However, the developing nations in particular should recognize the powerful potential of mPCR as a cost-effective, high-throughput means to improve their overall quality of life and living conditions. The most benefit can be obtained by focusing the initial efforts to develop practical mPCR methods on targeting pathogens with clinical and epidemiological relevance for the particular nations. Undoubtedly, the demands of rigorous standardization and validation to ensure the success of newly developed mPCR methods will be daunting to some, but the overall outcome will strengthen the nation's infrastructure by protecting and promoting its citizens' well-being.

Conflict of interest statement

I declare that I have no conflict of interest.

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