Editorial

Real-time PCR and the ultimate quest for real-time results

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"To realize its full potential, real-time PCR needs to be able to deliver real-time results."

It is likely that few readers of this editorial had the experience, in the few years after the principles of the PCR were announced in 1985, of actually performing the method as it was first described [1]. In those days, PCR involved pipetting a new aliquot of Klenow polymerase into the reaction tube after each PCR cycle because the temperatures required for denaturation of the target and amplification products also inactivated the enzyme. Most memorably, it required sequential precise pipetting of the reagents into small batches of plastic tubes wedged into styrofoam rafts floating in water baths kept at three different temperatures, with no time for bio-breaks. As graduate students, we were blissfully unaware of the threat of PCR contamination, the management or mismanagement of which ultimately determined the fate of the technology in forensic [2] and medical practice [3,4]. Fortunately, the technology evolved; with the advent of thermostable DNA polymerases, thermal cyclers and real-time PCR; contamination risk was diminished, physical containment requirements were reduced and more laboratories adopted molecular testing [5].

Real-time PCR testing has provided new levels of diagnostic accuracy and medical utility, particularly in the diagnosis of sexually transmitted diseases and protracted viral infections such as HIV and hepatitis C virus. Quantitative molecular methods have become the mainstay of the medical management of chronic viral infections, and will be used increasingly for monitoring treatment responses of a wide variety of infections [6]. Molecular typing methods will be used in real-time to track outbreaks of infections due to healthcare-associated pathogens [7]. Deep sequencing has facilitated metagenomic analysis of multiple prokaryotic pathogens within a biological ecosystem, thus defining diseases associated with shifts in bacterial populations such as inflammatory bowel disease and bacterial vaginosis [8]. Testing in this area may ultimately have bacterial ecology as its focus. From a

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