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Preface

Some readers of this book may have had the experience, in the few years after the 1985 announcement of PCR, of actually performing the method as it was first described by Kary Mullis. The procedure involved pipetting of new aliquots of Klenow polymerase 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 steps of floating small plastic tubes in water baths kept at three different temperatures, with no time for bio-breaks. The advent of a thermostable DNA polymerase was a dramatic improvement, but the novelty of the water baths quickly wore off, even for the most dedicated graduate student. I recall the great excitement at the University of California on the day when a prototype Perkin-Elmer thermal cycler was delivered to Dr. Jane Gitshier’s laboratory; one thermal cycler was placed in service of the entire university. The sign-up list quickly filled up as eager students, at all hours, filed into and out of her laboratory to perform experiments. The inconvenience of a midnight PCR run was a much-preferred alternative to standing, lock-kneed with pipettor in hand, in front of three water baths. Needless to say, with the advent of closed system detection and real-time PCR, times have changed.

Much of the subject matter of this book is focused on the implementation of these techniques for routine use in both clinical and research laboratories. In this volume lies a substantial repository of collective wisdom regarding the implementation, evaluation, and quality control of molecular diagnostic tests as they are currently available. From the standpoint of diagnostic test development, the major hurdles to be overcome are now related less to the nucleic acid detection technology itself than to sample processing. For years, clinical microbiologists, more than any other diagnosticians, have been tasked with gleaning diagnostic value from an incredible array of sample types, including stool, blood, pus, sputum, urine, tissues, and swabs from virtually every body site. Indeed, a significant impediment to implementation of molecular methods in this area has been to determine the appropriate quantity of specimen, how to concentrate the targets, how to release efficiently the target from its nearly impermeable shell, and how best to eliminate inhibitors. To paraphrase a once-famous opening line, “Sputum, the Final Frontier”; for the molecular diagnosticians focused on the challenge of rapidly detecting drug-resistant tuberculosis or ventilator-associated pneumonia, it truly is.

Other sections of this book provide a glimpse of the incredibly exciting future; since this future is inextricably linked to steps taken in the present day, we can expect to see incremental growth in several areas. 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. Molecular typing methods will be used in real time to track outbreaks of infections due to health care-associated pathogens. Deep sequencing has facilitated metagenomic analysis of multiple prokaryotic pathogens, thus defining better those diseases associated with shifts in bacterial populations such as inflammatory bowel disease and bacterial vaginosis. Testing in this area may have bacterial ecology as its focus. As diagnosticians, it seems likely that as the field evolves, so will our job descriptions. Fortunately for us, the days ahead seem exceedingly bright.

Still, much progress remains to be made. To press the Star Trek analogy further, what the universe needs now is the diagnostic equivalent of the Tricorder: a device or approach that can ascertain a patient’s condition comprehensively so that well-informed treatment and management decisions can be made in real time. Several sections of this book (in particular section VIII, Molecular Detection and Characterization of Viruses, and section XI, Systems Microbiology) illustrate how molecular diagnostics—as the first truly universal detection platform for bacteria, viruses, fungi, and protozoa—can be used to ask open-ended diagnostic questions about disease etiology. Until fairly recently, the decision to order a molecular diagnostic test has been prejudicial, in which the test is ordered on the basis of clinical likelihood of a “hit,” or positive result. In this setting, negative results often reflect nothing more than a clinician’s poor fortune in choosing among possible culprits. Multiplexed molecular techniques change that for-patient’s poor fortune in choosing among possible culprits. Multiplexed molecular techniques change that for-
in guiding everyday medical management decisions. It seems that once we start multiplexing and stop our batch-
ning, real-time PCR will finally be able to live up to its name.

As of the date of publication of this book, only about half of the high-complexity laboratories in the United States and European Union do molecular diagnostic testing of any kind, and most of these laboratories are limited to performing kit-based testing for chlamydia and/or gonorrhea. Relatively few laboratories still do their own test development and validation, which means that many tests with potentially high clinical impact are relatively inaccessible due to high cost or to prolonged turnaround times that make the results irrelevant. Outside the United States, the situation is even more bleak. Developing countries generally do not have access to molecular diagnostic technologies, despite the fact that these technologies could have an enormous impact on the health challenges of the developing world. In essence, molecular diagnostic testing is gaining ground rapidly, except in places where it is needed the most. It is hoped that the continued democratization of molecular diagnostics technology and the dissemination of privileged information, some of which is contained in the pages of this book, will help to correct this disparity.

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