PCR Detection of Bacteria in Seven Minutes

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Commercial spectrophotometric thermal cyclers are not ideally suited for on-the-go testing of samples because of a number of limitations, particularly with respect to size, weight, power usage, speed, and ruggedness. We have been developing silicon chip-based thermal cyclers that overcome these limitations. The Advanex Nucleic Acid Analyzer (ANAA), which has been recently described (8), consists of a user-friendly field and offers real-time power usage (for barcode and moving optical components). Samples are housed in a protective casing with each reaction module comprising a silicon reaction chamber with highly efficient integral thin-film heaters and a dedicated low-power optical system. The ANAA probe is monitored with blue light-emitting diode as an excitation source and two photodiode detectors with bandpass filters centered at 530 and 590 nm wavelength. When a real-time analysis algorithm detects a positive signal, the software will automatically inform the user via an audible alert and green-to-red indicator. 

Recent modifications made on the ANAA to enhance its performance led us to examine the time required to perform the TaqMan assay. Erwinia herbicola, a vegetative bacteria that serves as a surrogate for a pathogenic microbe such as Yersinia pestis (plague), was used in this study. Reactions containing 500 colony forming units (cfu) of Erwinia cells were mixed with a detection reagent, and the time required to perform the assay was recorded. The results showed that the time required for the ANAA to detect the presence of Erwinia cells was significantly shorter than that required for the TaqMan assay. This finding suggests that the ANAA could be a useful tool for rapid detection of bacteria in the field.
subjected to a series of thermal cycle settings (Table 1) ranging from our standard cycle time of 38 s to a reduced time of 17 s. Each run on the ANAA consisted of an initial heating at 96°C for 15 s to lyse the cells, followed by two-temperature cycling between 96° and 56°C. The detection profile (Fig. 2A) acquired with the 38-s cycle (Table 1) was characteristic for 500 Erwinia cells based on extensive quantification studies and blind field tests performed at the Joint Field Trials at Dugway Proving Grounds in Utah (9). After 24 cycles, or 15 min, a positive signal was clearly evident and was reported by the software. The displayed signal profile represented the collected raw data that was neither processed nor smoothed. The uniform baseline of the profile was attributed to the high signal-to-noise ratio.

Although 15 min was considered to be rapid detection, the limits of the real-time PCR assay had not been reached. Therefore, runs were performed with decreasing step times until a thermal cycling time of 17 s was attained (Table 1). At this short cycle time, the rate of thermal cycling was limited by the sample heating and cooling rates of 6.5° and 4.3°C per s, respectively. The temperature profile of the solution remained very consistent from cycle to cycle, and temperature control was maintained within 0.5°C at each step temperature (Fig. 3). The faster cycling produced a strong positive signal, which was automatically altered by the ANAA after just 7 min had elapsed (Fig. 2A). Only a small deleterious effect on the signal profile was observed, with the threshold cycle delayed by two cycles and the signal amplitude reduced by 16% compared to the 38-s cycle time.

To show that the assay was still quantitative under our most rapid conditions, reactions containing 500, 50, and 5 bacteria cells were tested with the 17-s cycle (Fig. 2B). Each 10-fold dilution resulted in an additional three to four thermal cycles or 1 min to detect the positive signal. This is exactly what was expected under conditions of very efficient PCR amplification where three to four cycles of exponential amplification would theoretically produce an 8- to 16-fold increase in the number of amplicons. The ability to perform quantitative assays under these conditions was due to (i) the efficient and uniform heating and cooling of the solution, (ii) the precision with which the target temperatures were reached and maintained (Fig. 3), and (iii) the sensitive optical system that only required 0.1 s to obtain a measurement at each cycle.

The results demonstrate that the total time to perform DNA analysis on a sample containing realistic concentrations of bacteria can be as little as 7 min with a highly efficient instrument consisting of inexpensive, low-power components. Total analysis time includes cell lysis, PCR detection of the PCR product with a target-specific FRET probe, and automated alerting of a positive signal. Recently, a flow-through PCR system has been described in which 20 cycles of PCR were performed in 90 s (70). Although this is an intriguing concept, the low efficiency of the reaction required the starting sample to contain 10^8 molecules of PCR product that had been obtained with a commercial air thermal cycler. The test actually represented a nested PCR, and the total time of analysis, including detection by agarose gels, was more than 1 hour.

Ultimately, the reaction kinetics of primer and probe hybridization combined with enzymatic chemistry must limit how short the cycle time can be while still maintaining good productivity per cycle. Until such a kinetic limit is reached, however, it is conceivable that the analysis time could be further reduced by developing more-efficient silicon chambers for faster ramping while maintaining precise temperature control, increasing the sensitivity of the detectors, and enhancing the thermal-stable polymerase activity by protein engineering. Silicon microstructures are also proving useful to rapidly purify and concentrate microbes and microbial DNA from complex mixtures (11, 12). This will permit ultrafast DNA testing for a wide variety of environmental and clinical samples.

References