Abstract

According to ASHI regulations temperature verification testing of all thermocyclers must be performed on a regular basis. Several testing mechanisms are recommended for the GeneAmp PCR System 9700 by the manufacturer. This report provides data which suggests that the MTAS system (Cyclertest, Landgraaf, The Netherlands) is a better method to assess the efficiency of amplification. Baseline testing was performed on a GeneAmp PCR System 9700 according to manufacturer specifications utilizing a GeneAmp PCR System 9700 Temperature Verification System Kit, a single probe system. This testing was within manufacturer specifications. The instrument, however, was performing sub-optimally in PCR-SSP typing according to several tray kit analyses. For our lot, 3040 (84%) trays run on this PCR had 5 or more dropouts, no control or specific amplicon was detected. The average number of dropouts per tray was 12 (12095 ± 35°). Testing this thermocycler with the MTAS system, a 16 probe system, by the licensed provider (Appropriate Technical Resources, Laurel, MD) revealed that this machine had an average temperature overshoot at 95°C of 97.92°C. This level of overshoot could lead to excessive degradation of sample, primers and exons leading to possible false readings. The MTAS system produces information which would not routinely be detected in standard temperature verification procedures; therefore, yielding additional insight into the performance of the instrument. The information provided by this system will likely be very useful in troubleshooting thermocycler performance in clinical laboratories.

Methods

Two separate methods were utilized to determine the performance of a GeneAmp PCR System 9700. Major points are described below:

- GeneAmp PCR System 9700 Temperature Verification System Kit utilizes a single probe system to perform three different tests.
- Temperature calibration verification test
- Temperature uniformity test
- System rate and cycle test
- The CYCLERtest system utilizes Multi Channel Dynamic Temperature Measurement System (MTAS). This is a multi channel temperature acquisition system.
- Measurements are performed statically as well as dynamically.
- Measurements of 16 channels simultaneously.
- Certificate of thermal validation

Discussion

In reviewing the information that was found utilizing the MTAS system, several points need discussion. The thermocycler in question was yielding results which were questionable for PCR-SSP typing. For one particular tray lot, 3040 (84%) trays run on the PCR machine in question had 5 or more dropouts (no control or specific amplicon detected). This rate was at a much higher rate than seen for this same lot on other thermocyclers in the laboratory. There also seemed to exist some consistency concerning which wells had dropouts. The temperature probe locations utilized for this test were kept constant. Figure 1 depicts the various channels which were used for this test. In this particular test, the various spots of concern were in the vicinity of the probe locations. The overall results of the CYCLERtest can be seen in Table 2. One of the most striking results discovered was the overshoot determined for this block. The maximum overshoot is described as the maximum value of all active sensor channels towards a static temperature plateau. Overshoot for this block is 98.98°C which is 3.98°C over the target temperature. This is depicted in Figure 3. Such a high temperature spike could create an issue with the denaturing of the sample as well as the possibility of breaking down the primers. In addition to these factors, the enzyme utilized in the reaction will begin to break down at a rate of 30% for every two degrees over the optimal target temperature. This particular instrument has potential problems at 60°C and 50°C. The undershoots at these temperatures can be depicted in Figure 2 in the Dynamic Overview of the instrument. Undershoot temperatures could play a factor in slowing the reaction too fast. For example, when the target temperature is 50°C after cooling from 90°C the maximum undershoot for this instrument was almost 5°C. Thus taking the target temperature of 50°C to 45°C. In addition to the static temperatures which are recorded by the MTAS, heating and cooling rates can usually be calculated via computer program equations depending on the instrument being tested. This particular instrument, a GeneAmp PCR 9700, has an internal program which changes the linearity of the slope generated by the cooling cycle. Due to this, it is difficult for the cool rate to be calculated for every temperature. Different thermocycler models may not have this problem. Other instruments which were tested in the laboratory yielded significant information concerning the heating and cooling rates of the instrument. When utilizing this system, all of the factors must be taken into consideration in order to determine whether an instrument is performing at optimal levels.

Summary

The MTAS system produces information which would not routinely be detected by standard temperature verification procedures. All of the information that was evaluated yields additional insight into the performance of the instrument. This information is beneficial in troubleshooting thermocycler performance.

Reference: MTAS system, CYCLERtest, Landgraaf, The Netherlands. Pictures and results were reproduced from the CYCLERtest Standard Report Format.