Design and testing of a convenient diagnostic tool to test BD MAX™ temperature sensors

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Introduction

Polymerase chain reaction (PCR) is the process of amplifying DNA for analysis. Real-time PCR is "the most powerful tool for quantitative nucleic acids analysis" (Kubista et al., 2006). During real-time PCR the amplification of the desired DNA is tracked using fluorescent dyes, then the amount of fluorescence is graphed against the number of heating and cooling cycles. This creates a melt curve that is used in the medical field to diagnose diseases and used in forensics to identify suspects. The target DNA's melt curve is compared to known melt curves created by specific diseases or specific suspects. The BD MAX™ is a fully integrated platform that can perform real-time PCR, meaning it performs automated extraction and thermocycling with a small amount of hands-on time needed. A high degree of precision is needed for extraction; automated extraction substantially decreases risk of contamination and does not require an operator to have a high level of precision. However, multiple BD MAX™ machines have been experiencing error messages that state that either the heaters or temperature sensors are malfunctioning, leading to inaccurate test results. For BD to diagnose the problem, a field engineer would be sent out to the malfunctioning MAX's location to take the heater mux board (a component that carries both the heaters and temperature sensors) to a calibration oven. On sight repair decreases instrument downtime, which leads to a more timely, and accurate result for the patient. The goal of the project was to create a convenient diagnostic tool to resolve error messages in the field. The tool had to heat uniformly to a known temperature and be placed on the mux board to evaluate the MAX's temperature sensors and heaters.

Materials and Methods

A closed water-heated recirculation system was engineered to continuously circulate water between a hotplate-heated water bath and an aluminum block. A Polylactic Acid (PLA) block was designed in Autodesk Inventor (Figure 1), and was three-dimensionally (3D) printed multiple times using a Dimension Elite 3D printer. Various functionality tests were performed on each block. The objective of the first functionality test was to fit the base of the block on the mux board of the BD MAX™. Once fitted, the block was tested for leakage (Figure 2). After successfully completing these tests, a new block with hose adapters was designed, and leakage testing was performed on the new block. From there, the design was refined until precision milling equipment could be used to machine the design. Alterations had to be made to machine the block. Ten threaded holes were strategically incorporated into the face of the block to bolt a gasket and polycarbonate sheet onto the block. Adapters could not be machined with the block, so holes were left at the entrance and exit of the water channels for brass adapters to be press fitted. The aluminum block with a gasket and polycarbonate sheet (Figure 3) were functionality tested for leakage.

After confirming that the aluminum block did not leak, temperature testing began. A FLIR® E5 Thermal Imaging Camera (TIC) was used to visually check for hot spots on the surface of the block. Surface temperature probes were used to measure the temperature at the corners and center of the block simultaneously at one-minute intervals for 50 minutes; trials were run in triplicate. Time started when the hotplate was turned to the lowest setting.

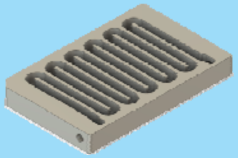


Figure 1: (right) A 4.375" × 2.806" × 0.500" 3D model of the final block design. Ten threaded holes were drilled on the top face to secure the rubber gasket and polycarbonate sheet to the block. Two ¼" brass adapters were press fitted to the water channel exit and entrance (circular hole on left face.)

1 inch

Figure 2: (left) A photograph of the first material that was tested as a gasket. This cork sheet did not seal the area between the block and polycarbonate sheet because the cork was too pliable. The thin pieces of cork did not align with the water channels.

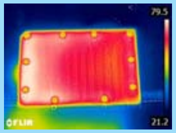
1 inch

Figure 3: (right) A photograph of the final block design being tested. The aluminum block used a rubber gasket opposed to the cork gasket pictured above.

Results

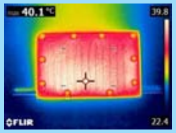
2 inches

Figure 4: Thermal image captured with the TIC at time 5 minutes (Trial 2).



2 inches

Figure 5: Thermal image captured with the TIC at time 30 minutes (Trial 2).



2 inches

Figure 6: Thermal image captured with the TIC at time 40 minutes (Trial 2).

The block reached a maximum of 79.5 °C in all three trials. Thermal images were captured every five minutes. The scale bar on the right side of these images varies as temperature in the image varies. The number in the top left of these images shows the maximum temperature at a point, this mode was disabled between 20 and 40 minutes because the block heated and cooled uniformly during that interval. This test was designed to measure the time it took to heat the block (Figure 4) to it's maximum temperature (Figure 5) and back down (Figure 6) to room temperature.

Conclusions

The surface of the block heated to a uniform temperature, but only reached 79.5 °C. This was because of excess aluminum on the bottom face. The goal was for the block to reach 95.0 °C. From the current stage, continued temperature testing must be done and there must be alterations to reduce heat loss, such as reducing the height of the block. The closer the block can get to boiling, the more accurate the test will be.

Once extensive data is collected and the block is machined with less excess aluminum on the bottom face, a program should be developed for the field engineer to determine if the difference between the known temperature of the block and the temperature readings given by the BD MAX™ are significant.

References

Kubista, M., Andrade, J. M., Bengtsson, M., Forootan, A., Jonak, J., Lind, K., . . . Zoric, N. (2006). The real-time polymerase chain reaction. *Molecular Aspects of Medicine, Volume 27*. Retrieved from http://www.multid.se/publications/TheReal-timePolymeraseChainReaction.pdf