

LightCycler Amplification and Detection of DNA and RNA Targets in Plastic Tubes

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Introduction/Background

Osmetech has developed a plastic tube as an alternative to the fragile glass capillaries used in the Roche LightCycler[®]. OPTI TUBES are made from an optically clear plastic, TOPAS[®], which has low intrinsic fluorescent background and a melting point that is above the temperatures encountered during PCR. The OPTI TUBE product consists of disposable OPTI TUBES and caps along with an OPTI TUBE Carousel that is designed for use in the LightCycler (Figure 1). OPTI TUBES and caps are compatible with the Roche Capillary Capping Tool and capillary centrifuge adapters. The OPTI TUBE Carousel is compatible with the Roche LightCycler Carousel Centrifuge.



Figure 1
OPTI TUBE and OPTI TUBE Carousel

OPTI TUBES were developed using a Factor V Leiden (FVL) research assay, which employs fluorescence-based HyBeacon[®] probe technology (1) to detect the FVL mutation (G1691A). However, the tubes are designed to accommodate a variety of PCR applications ranging from SNP, point mutation, and DNA deletion detection to DNA and RNA sequence detection of infectious disease agents. In addition, the tubes must be compatible with different detection strategies, such as end-point FRET and real-time TaqMan[®] detection.

In the following study, the performance of OPTI TUBES was compared to glass capillaries with commercially available PCR research kits encompassing different amplification and detection strategies.

Materials and Methods

Three commercial PCR test kits were obtained for use in this study. Each kit and the corresponding procedure for the kit are described below.

The Applied Biosystems CYP2C19*2 kit is used for detection of the point mutation G681A in the relevant Cytochrome P450 gene. It is a TaqMan real-time detection based assay that uses an internally quenched dual-labeled probe for detection. The assay begins with an UNG pre-incubation step at 50 °C for 600 sec. The PCR reaction is a two temperature PCR of 50 cycles; 92 °C for 15 sec and 60 °C for 90 sec. The appearance of the TaqMan fluorescence signal was used to determine the presence of the CYP2C19*2 mutation. A total of 28 samples were tested with the glass capillaries and OPTI TUBES. Genomic DNA control samples containing 200, 20, and 2 ng DNA were tested in triplicate and 0.2 and 0.02 ng controls were tested at n=5. Positive and negative controls were tested in triplicate.

The Roche ApoE kit is used for the detection of the point mutations in codons 112 and 158 of the human Apolipoprotein E gene. The test is a FRET-based assay with dual probes for end-point detection. The PCR reaction was 45 cycles of 95 °C for 0 sec, 60 °C for 10 sec, and 72 °C for 10 sec followed by a melt from 42-80 °C at a ramp rate of 0.1 °C per sec. A total of 12 samples were tested with the glass capillaries and OPTI TUBES; 10 samples purified from blood and the kit positive and negative controls.

The Artus Influenza LC RT-PCR kit is used for the detection of the RNA from the influenza A/B viruses. The test is a real-time reverse transcriptase (RT)-based PCR assay that uses real-time detection technology. The PCR reaction began with a RT step at 50 °C for 600 sec, followed by a denaturation step and 45 cycles of PCR at 95 °C for 2 sec, 55 °C for 12 sec, and 72 °C for 10 sec. A total of 12 samples were tested with glass capillaries and OPTI TUBES. Purified inactivated Flu A RNA (kindly provided by Dr. Greg Chiklis, Zepmetrix, Corp.) was tested at the following dilution levels at n=2: neat, 1:10, 1:100, 1:1000, 1:10000. The kit positive and negative controls were included at n=1 in the assay runs.

Results

CYP2C19*2 Kit

Both glass capillaries and plastic OPTI TUBE dilution series yielded linear standard curves (Figure 2 and Figure 3, respectively). The variability in the 0.2 ng (~120 DNA copies) standards was evident in both plastic and glass and is interpreted as sample dilution variability, not PCR variability. The crossover points in plastic were typically at earlier PCR cycles (as much as 9 to 10 cycles earlier) than in glass. This would suggest that this PCR is more efficient in plastic tubes than in glass. In contrast the slope sensitivity of the standard curve was about 30% less in plastic than in glass.

ApoE Kit

When the recommended protocol was followed, the assay performed as expected in glass but failed to yield melt peaks with OPTI TUBES. Because plastic has a known slower thermal transfer rate than glass, it was hypothesized that the dwell time of 0 seconds at 95 °C was a likely cause of the weak amplification. Therefore the dwell time for amplification in OPTI TUBES was changed to 10 seconds and the procedure was carried out as before with the lengthened denaturation time. This assay yielded satisfactory results as shown in Figure 4.

The assay for codon 112 showed well-resolved melt peaks in heterozygous samples in both glass and OPTI TUBES (Figures 4 and 5). Comparison of melt temperatures obtained in glass capillaries versus OPTI TUBES was carried out. There was 100% genotype agreement between glass and OPTI TUBES and a T_m comparison between glass capillaries and OPTI TUBES revealed a 0.4 and 0.8 °C T_m difference for T_{m1} and T_{m2} , respectively, with excellent T_m reproducibility (all examples exhibited less than 0.2% C.V. in glass versus less than 0.1% C.V. in plastic). The peak separation was 6.5 versus 6.2 °C (glass versus plastic).

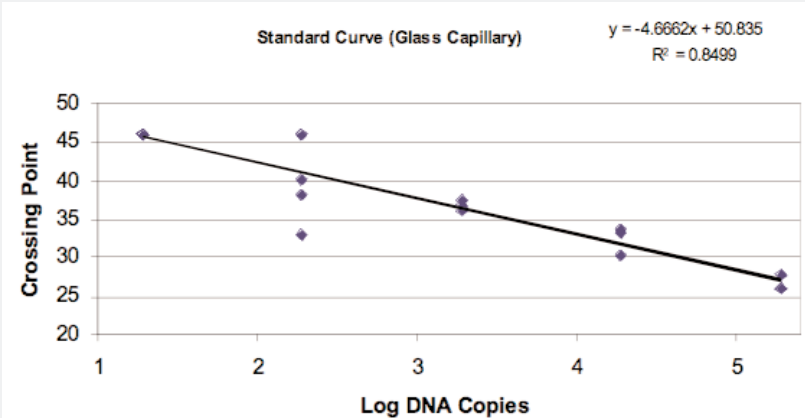


Figure 2
CYP2C19*2 Standard Curve in Glass Capillaries

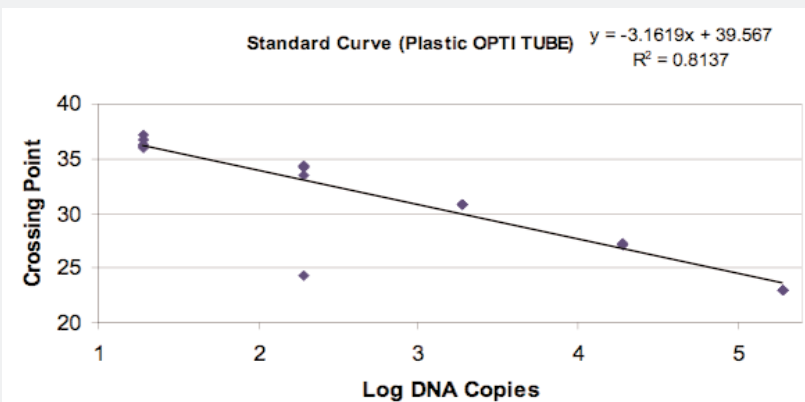


Figure 3
CYP2C19*2 Standard Curve in OPTI TUBES

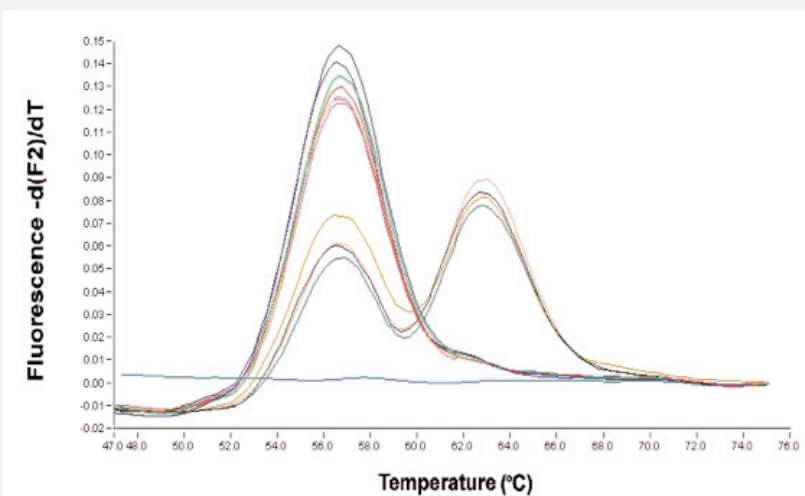


Figure 4
ApoE Codon 112 Melt Peaks: OPTI TUBES

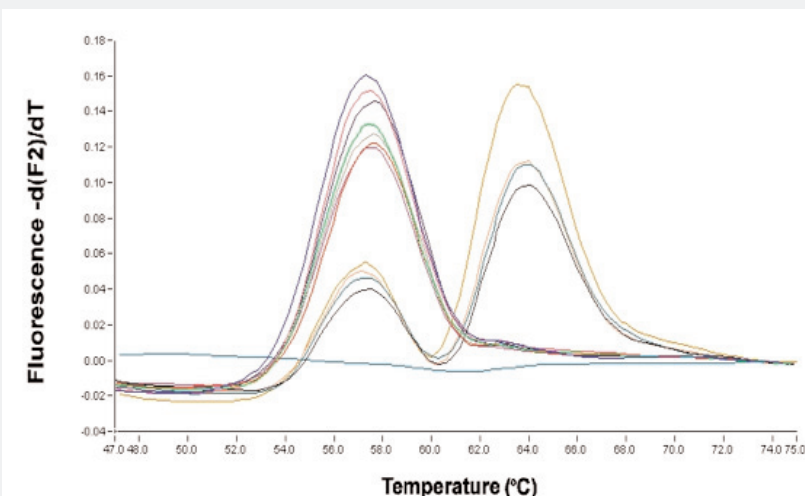


Figure 5
ApoE Codon 112 Melt Peaks: Glass Capillaries

The simultaneous assay for the codon 158 mutation showed well-resolved melt peaks in heterozygous samples in both glass and plastic OPTI TUBES. Again, a 100% genotype agreement was observed between glass and OPTI TUBES. The T_m comparison between glass capillaries and OPTI TUBES revealed a 0.4 and 0.2 °C T_m difference for T_{m1} and T_{m2} , respectively, with good T_m reproducibility (all examples exhibited less than 0.4% C.V. in glass versus less than 0.4% C.V. in plastic). The peak separation was 7.3 versus 7.4 °C (glass versus plastic).

Flu A/B Kit

Both glass capillaries and OPTI TUBES dilution series yielded linear standard curves. The average crossover points in OPTI TUBES and capillaries were within 1.5 cycles of each other at all dilutions except the 1:10,000 dilution where the crossover point in OPTI TUBES was 2-3 cycles later than in glass. The slope sensitivity of the standard curve in OPTI TUBES was 30% greater than in glass, and in both cases the reproducibility of the test was good as evidenced by the close replicate values plotted on the standard curves (Figures 6 and 7).

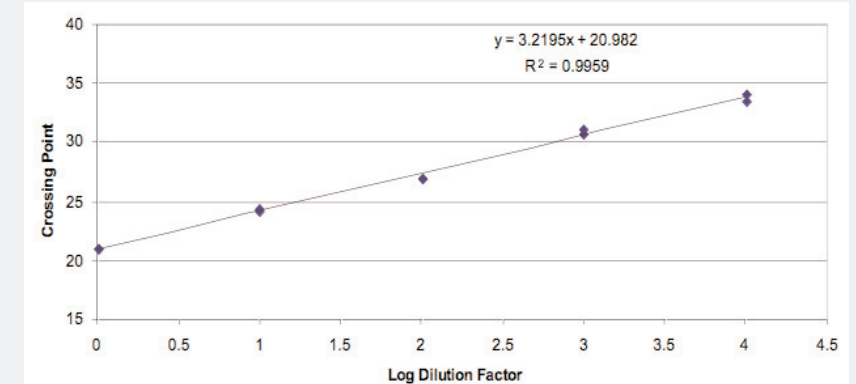


Figure 6
Flu A RNA Dilution Standard Curve in Glass Capillaries

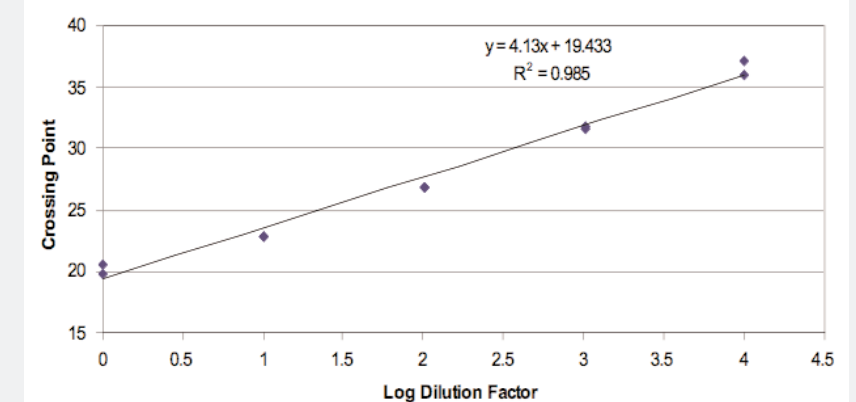


Figure 7
Flu A RNA Dilution Standard Curve in OPTI TUBES

Conclusions

OPTI TUBES showed equivalent performance to glass capillaries in three different applications, and show promise to mitigate the risks associated with broken glass fragments and potential amplicon contamination. These studies make no claim regarding clinical performance of the assays tested but provide evidence that plastic tube substitution is not expected to have a major technical impact on assay function.

As exemplified by the ApoE test data, optimal PCR conditions may differ between OPTI TUBES and glass capillaries and, as with any new experimental system, the user is recommended to test and optimize their specific application when transferring to OPTI TUBES.

References

1. French, DJ, Archard CL, Brown T, McDowell D. HyBeacon[™] probes: a new tool for DNA sequence detection and allele discrimination. Mol. Cell. Probes 2001; 15:363-74.