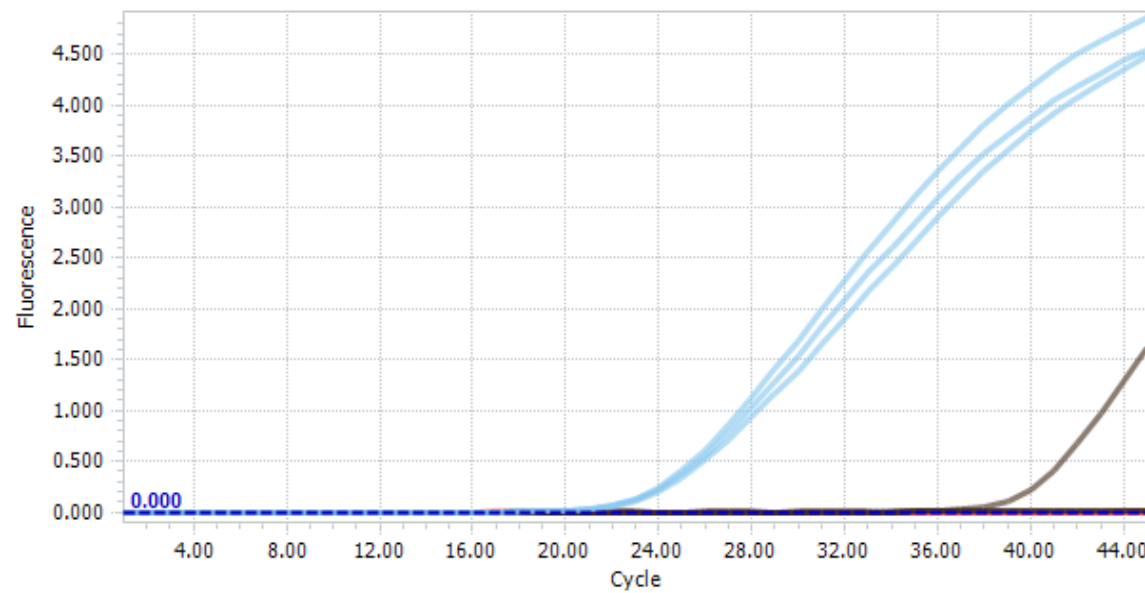
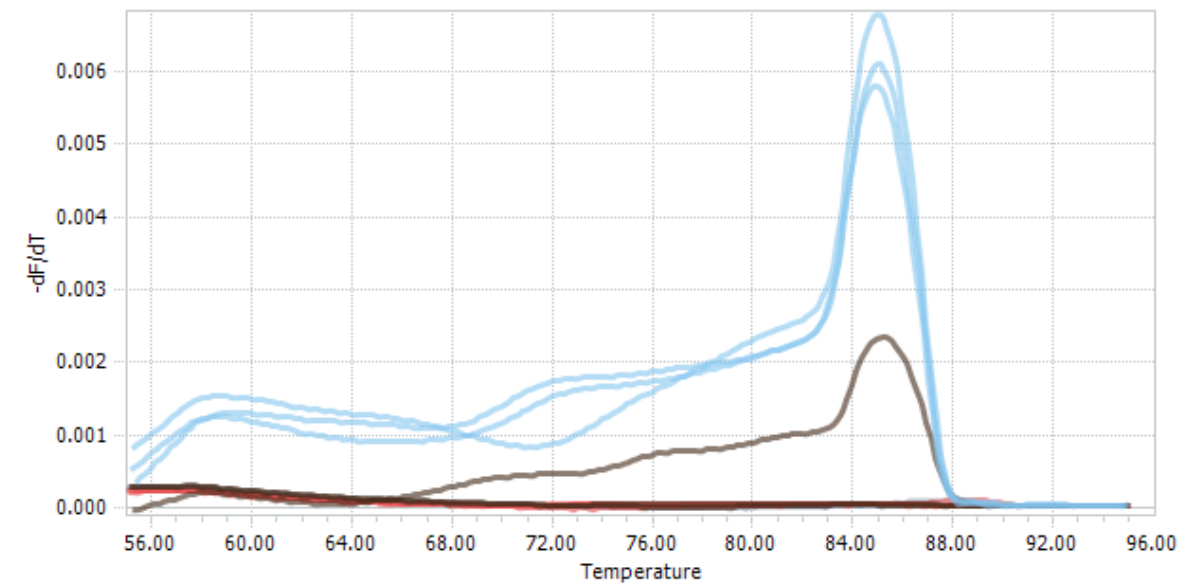


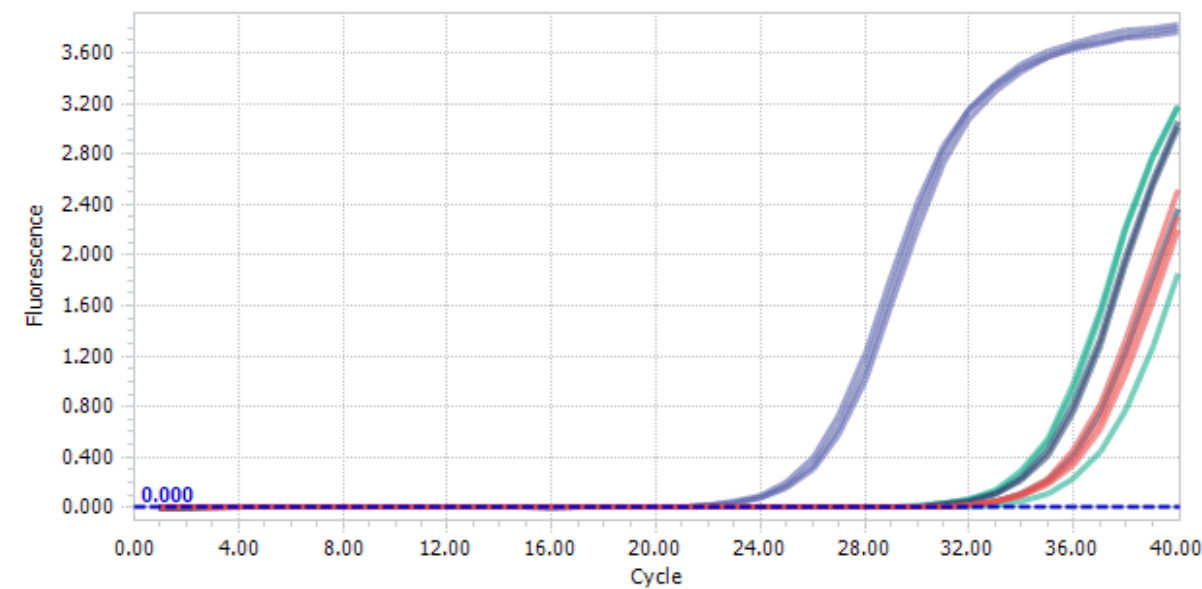
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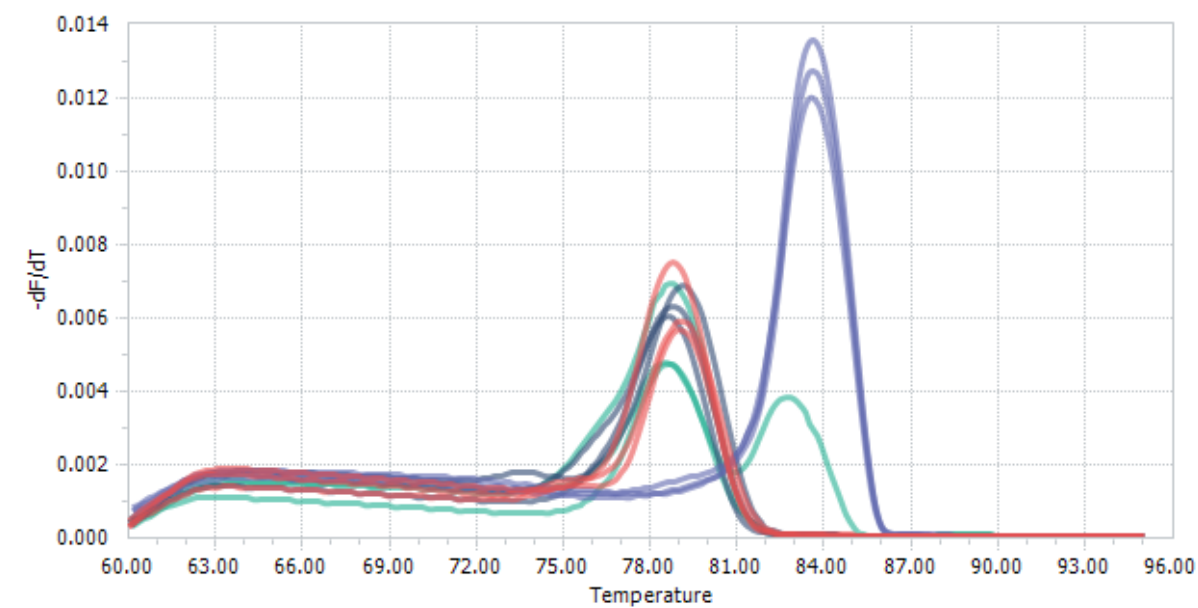
B



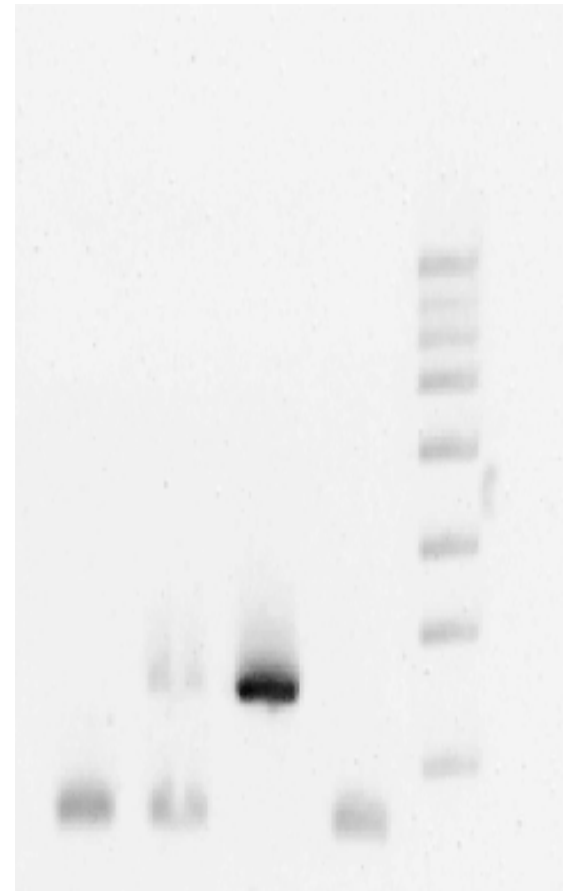
C



D



E



#### Supplementary Figure 5. Results of HSV-2 and HIV-1 specific PCR

Amplification curve analysis demonstrated that CSF from subject M029 (blue, positive control) amplified in three out of three replicates (mean Ct = 23.8), consistent with positive mNGS results for HSV-2. CSF from subject M132 (gray) amplified in only one out of three replicates (Ct = 39.8); correspondingly, no HSV-2 reads were detected by mNGS. There was no amplification from the negative control (red) (A). Melting curve analysis demonstrated consistent curves across all positive wells (B). Amplification curve analysis demonstrated that CSF from subject M061 (purple, positive control) amplified in three out of three replicates (mean Ct = 25.2), consistent with positive mNGS results for HIV-1. CSF from subjects M051 (blue) and M010 (gray) amplified at high Ct values, similar to the negative control (red) (C). Melting curve analysis demonstrated that only one replicate from M051 melted in a pattern consistent with the positive controls; the other positive wells melted at lower temperatures, suggestive of nonspecific amplification or primer-dimerization (D). Gel electrophoresis results from PCR products demonstrate a band of the expected size for subject M061 (positive control) and a faint band of the expected size for subject M051, but not M010 or the negative control (E).