Study of PCR inhibitors on HiMedia's Insta Q96®-6.0 Real-Time PCR System for Forensic Applications

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Introduction

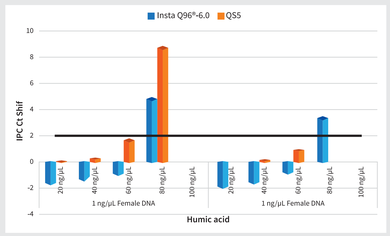
HiMedia's Insta Q96®-6.0 Real-Time PCR system is an open, exclusive system for forensic applications, enabling real-time quantitative PCR for amplification of short tandem repeats (STR) and downstream analysis. This six-color optical filter set provides precise and accurate DNA quantification, allowing users to select reagents and test kits without calibration. HiMedia's Insta Q96®-6.0 Real-Time PCR system requires checking amplification against PCR inhibitors, following SWGDAM's guidelines for optimal performance.

Objective

The objective of this study is to check the efficiency of HiMedia's InstaQ96®-6.0 Real-Time PCR System at different concentrations of humic acid and hemin.

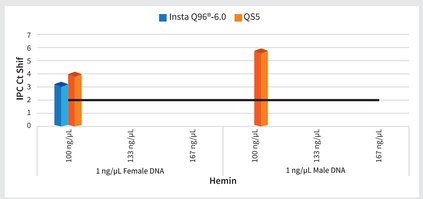
Results

Figure 1: IPC Ct Shift results from 1 ng/µL male and 1 ng/µL female DNA mixed with increasing amounts of humic acid



The X-axis represents the DNA sample and amount of humic acid. The Y-axis represents the IPC Ct shift. The black line represents the default IPC Ct threshold of 2. Absence of column bar in either of the real-time PCR system indicates complete inhibition.

Figure 2: IPC Ct Shift results from 1 ng/µL male and 1 ng/µL female DNA mixed with increasing amounts of hemin



The X-axis represents the DNA sample and amount of humic acid. The Y-axis represents the IPC Ct shift. The black line represents the default IPC Ct threshold of 2. Absence of column bar in either of the real-time PCR system indicates complete inhibition.

Figure 3: Average concentration of small autosomal target exposed to increasing amounts of humic acid and hemin mixed with 1 ng/µL female DNA and 1 ng/µL male DNA

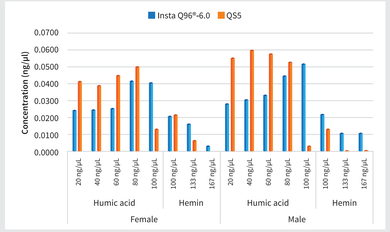


Figure 4: Average concentration of large autosomal target exposed to increasing amounts of humic acid and hemin mixed with 1 ng/µL female DNA and 1 ng/µL male DNA

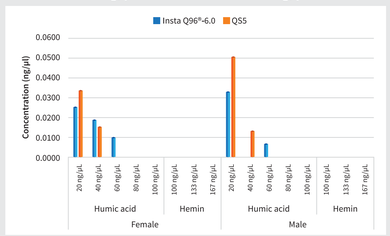
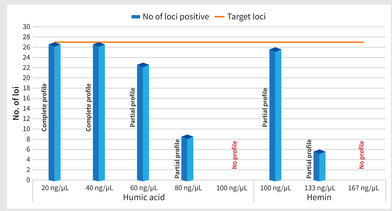
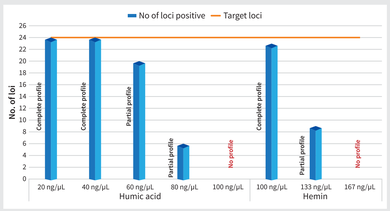


Figure 5: Total loci amplified to increasing amounts of humic acid and hemin mixed with 1 ng/µL male DNA



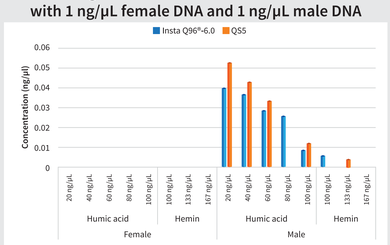
STR Profiling performed using Promega's VersaPlex® 27PY System on a 3500XL CE instrument

Figure 6: Total loci amplified to increasing amounts of humic acid and hemin mixed with 1 ng/µL female DNA



STR Profiling performed using Promega's VersaPlex® 27PY System on a 3500XL CE instrument

Figure 7: Average concentration of male target exposed to increasing amounts of humic acid and hemin mixed with 1 ng/µL female DNA and 1 ng/µL male DNA



Female DNA showed no amplification for male target

Figure 8: Amplification of 1 ng/µL male DNA

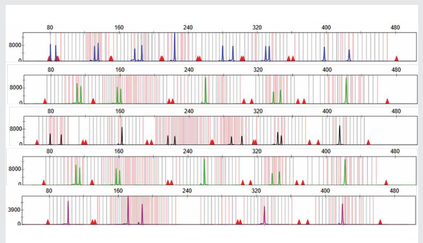
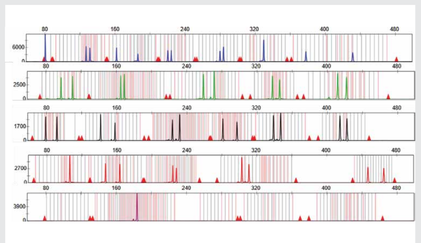
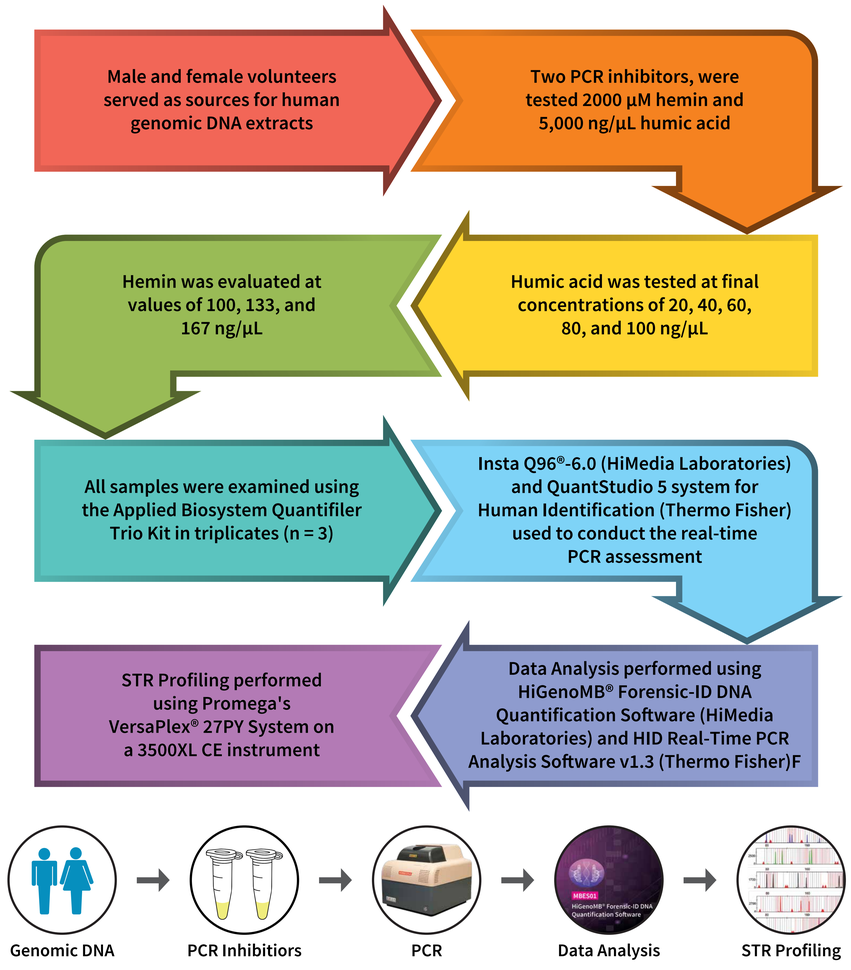


Figure 9: Amplification of 1 ng/µL female DNA



Materials and Methods



Conclusion

1. The internal positive control Ct (IPCCT) shift was seen in comparison to the control DNA.
2. IPCCT was flagged for humic acid concentrations of 80 ng/µL, 100 ng/µL and all three concentrations of hemin.
3. **HiMedia's Insta Q96® - 6.0 Real-Time PCR System showed early detection of 1.5 Ct in comparison to Applied Biosystem's QuantStudioTM 5 Real-Time PCR System.**
4. **The results demonstrate that HiMedia's Insta Q96®-6.0 Real-Time PCR System is capable of amplifying DNA at different concentrations of PCR inhibitors and that the system is suitable for use within a forensic laboratory.**

References

1. Validation Guidelines for DNA Analysis Methods – Scientific Working Group on DNA analysis Methods. https://docs.wixstatic.com/ugd/4344b0_813b241e8944497e99b9c45b163b76bd.pdf.
2. Quantifiler® HP and Trio DNA Quantification kits User Guide, https://assets.thermofisher.com/TFS-Assets/LSG/manuals/4485354.pdf.
3. VersaPlex™ 27PY System for Use on the Applied Biosystems® Genetic Analyzers. https://www.promega.in/-/media/files/resources/protocols/technical-manuals/tmd/tmd055-versaplex-27py-system-technical-manual.pdf?rev=012be1a030d34ee1937688139276118&sc_lang=en