World Academy of Science, Engineering and Technology
International Journal of Medical and Health Sciences
Vol:19, No:06, 2025

Lab-on-Chip Multiplexed qPCR Analysis Utilizing Melting Curve Analysis Detects Up to 144 Alleles with Sub-hour Turn-around Time

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Abstract : Rapid genome testing can provide results in at best hours to days, though there are certain clinical decisions that could be guided by genetic test results that need results in hours to minutes. As such, methods of genetic Point of Care Testing (POCT) are required if genetic data is to guide management in illnesses in a wide variety of critical and emergent medical situations such as neonatal sepsis, chemotherapy administration in endometrial cancer, and glucose-6-phosphate dehydrogenase deficiency (G6PD)-associated neonatal hyperbilirubinemia. As such, we developed a POCT “lab-on-chip” technology capable of identifying up to 144 alleles in under an hour. This test required no specialized training to utilize and is suitable to deployment in clinics and hospitals for use by non-laboratory personnel such as nurses. We developed a multiplexed qPCR-based sample-to-answer system with melting curve analysis capable of detecting up to 144 alleles utilizing the Kelliop RapidSeq126 PCR platform combined with a single-use microfluidic cartridge. The RapidSeq126 is the size of a standard desktop printer and the microfluidic cartridges are smaller than a deck of playing cards. Thus the system was deployable in the outpatient setting for clinical trials of MT-RNR1 genotyping. The sample (buccal swab from volunteers or plasmids in media) used for DNA extraction was placed in the cartridge sample inlet prior to inserting the cartridge into the RapidSeq126. The microfluidic cartridge was composed of heat resistant polymer with a sample inlet, 100um conduits, liquid and solid reagents, valves, extraction chamber, lyophilization chamber, 12 PCR reaction chambers, and a waste chamber. No human effort was required for processing the sample and performing the assay other than placing the sample in the cartridge and placing the cartridge in the RapidSeq126. The RapidSeq126 has demonstrated ex vivo detection in plasmids and in vivo detection from human volunteer samples of up to 144 alleles per microfluidic cartridge used and did not require specialized laboratory training to operate. Efficacy was proven for several applications, such as multiple microsatellite instability (MSI) sites (SULF/RYR3/MRE11/ACVR2A/DIDO1/SEC31A/BTBD7), endometrial cancer POLE exonuclease domain (EMD) mutation status, and G6PD variants such as those commonly associated with hemolysis (c.202G>A, c.376A>G, c.680G>A>T, c.968T>C, 404A>C, c.871G>A). The RapidSeq126 system was also able to identify the three MT-RNR1 variants associated with aminoglycoside-induced sensorineural hearing loss (m.1555A>G, m.1095T>C, m.1494C>T). Results were provided in under an hour in a sample-to-answer fashion requiring no processing other than inserting the cartridge with the sample into the RapidSeq126. Results were provided in a digital, HL7-compliant format suitable for interfacing with Electronic Healthcare Record (EHR). The RapidSeq126 system provides a solution for emergency and critical medical situations requiring results in a matter of minutes to hours. The HL7-compliant data format of results enables the RapidSeq126 to interface directly with EHRs to generate best practice advisories and further reduce errors and time to diagnosis by providing digital results.

Keywords : genetic testing, pharmacogenomics, point of care testing, rapid genetic testing
Conference Title : ICP 2025 : International Conference on Pediatrics
Conference Location : San Francisco, United States
Conference Dates : June 12-13, 2025