Rapid Molecular Detection of Adenosine Deaminase Deficiency Severe Combined Immunodeficiency Using High-Resolution Melt Analysis

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Severe combined immunodeficiency (SCID) is a group of rare genetic disorders that causes affected individuals to have little or no immune response due to a deficiency or absence of T-cells and at least one other type of lymphocyte. This leaves SCID patients susceptible to persistent bacterial, fungal, and viral infections which can potentially be lethal. SCID patients need to be diagnosed early on in order to receive effective treatment. Our group's assignment was to design, optimize, and validate an assay that could accurately genotype for adenosine deaminase (ADA) deficiency SCID. We were given six weeks and enough reagents to test 400 samples. Primers were designed to produce a small target amplicon of 71bp containing the rs121908723 single nucleotide polymorphism (SNP) within the *ADA* gene. SCID homozygous and SCID heterozygote Coriell Cell Repository DNA controls were used to design and validate a qualitative SCID high-resolution melt (HRM) genotyping assay using the Rotor-Gene Q platform. Isolated DNA is amplified by real-time polymerase chain reaction (PCR), and the target amplicon is fluorescently detected using EvaGreen dye during HRM analysis. HRM has the ability to distinguish samples by a fraction of a degree difference and a single base pair change, which is the case in ADA SCID. A total of 147 samples consisting of the SCID homozygous and heterozygote from Coriell Cell Repository and de-identified isolated patient DNA were analyzed. The results provided the data necessary to measure analytical and clinical performance, lower limit of detection, and analytical specificity. The assay demonstrates a promising analytical and clinical performance with an accuracy of 92.6%, a precision of 92.2%, a clinical sensitivity of 90.8%, a clinical specificity of 100%, a positive predictive value of

100%, and a negative predictive value of 95.7%. With additional validation studies, our assay has the potential to be used clinically for the rapid genotyping of possible SCID patients.