Methods of Quantifying Antiviral Drug Resistant Mutations in Influenza A

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Technology description

Researchers at TGen and Northern Arizona University (NAU) have developed a real-time allele specific mixture analysis (ASMA) qPCR assay for quantitatively analyzing the amount of antiviral drug resistant Influenza A mutants in a mixed viral sample. This highly sensitive method provides a cost effective tool for clinical or public health laboratories to quantitate <0.1% SNP minor components in mixed Influenza A samples.

The rise of antiviral resistance in influenza poses a major challenge to influenza pandemic preparedness. With the limited treatment options of adamantanes and neuraminidase inhibitors as the only FDA approved drug classes, rapid and sensitive resistance monitoring methods become of increased importance for combating influenza pandemics. Commonly used sequencing platforms for monitoring resistance-conferring mutations in influenza suffer from sensitivity limitations when analyzing mixed viral populations, with some resorting to visual evaluation techniques such as electropherogram to compensate in the quantification of minor components. Single-nucleotide mutations that confer resistance via a single amino acid substitution in influenza are well-characterized and have been used effectively as markers in monitoring resistance, but cannot be accurately quantified by these methods with sensitivity limitations.

ASMA qPCR provides a rapid, sensitive, and highly quantitative method for targeting resistance conferring SNP minor components in high throughput applications by incorporating one or more mismatches in a selective primer either near or at the SNP. The TGen and NAU developed real-time ASMA qPCR assay was validated using the Influenza A M2 gene mutations L26F, V27A, A30T, and S31N; and the NA gene mutation H274Y, which are known to confer resistance to antiviral drugs but the assay may be adapted for use with a much larger library of mutations. Using this assay, the mutant load in a mixed viral sample can be characterized using the ratio of detected mutants to detected wild types. The high sensitivity of this ASMA qPCR assay in very low levels of SNP minor components has the potential to enable early and prospective monitoring of emerging resistance in drug development, public health, and clinical settings. This ASMA qPCR assay may also have further utility in fields such as microbial forensics, human cancer characterization, and the study of mixed communities in diverse organisms, where resolution beyond traditional SNP genotyping analysis provides added value.

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