The Dose Response and Abstinence induced Reversion Curves for Cigarette and Alcohol Consumption using Clinically Implementable Droplet Digital PCR Assays

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Recent studies have shown that self-report of smoking or drinking in high risk populations is unreliable. This misclassification or underestimation is a profound barrier to the formulation of effective treatment and interventions for substance use disorders and substance use related medical conditions. Conceivably, DNA methylation approaches could overcome this hurdle. Specifically, using genome wide approaches, we and others have shown that both smoking and drinking induce substance specific, potentially reversible changes in DNA methylation. Unfortunately, these systematic approaches are expensive to conduct and difficult to translate. To overcome these barriers, this past December and October, using a droplet digital PCR (ddPCR) implementation process, we introduced sensitive and specific, potentially clinically translatable ddPCR assays (Smoke Signature© and Alcohol Signature™) that are capable of detecting cigarette and heavy alcohol consumption with extremely high accuracy (0.99 and 0.98 AUC, respectively). Now, using the data from 300 subjects whose self-report of smoking status was confirmed with exhaled CO and cotinine determinations, we show the 1) dose dependent relationship between cigarette consumption and DNA methylation status at cg05575921 (Smoke Signature©) and 2) the 30 day reversion curve of DNA methylation in response to smoking cessation. In addition, with respect to alcohol consumption, we present the initial shape of the dose response curve and the 30 day reversion curve for methylation in response to alcohol abstinence. We conclude that ddPCR assessments of DNA methylation status can accurately impute the presence and degree of smoking and alcohol consumption behaviors. In direct contrast to existing methods, these assessments can be conducted in individual laboratories and their results can now be easily translated into quantities consumed for research purposes, and in the future, into potentially actionable clinical data. We suggest that the adoption of these approaches could lead to significant improvements in the diagnosis and treatment of substance use disorders.