# Quantitative DNA Methylation Analyses Using Digital Droplet PCR Sensitively Detects Surreptitious Smoking in Adolescents.

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Effective interventions to prevent the initiation and escalation of smoking do exist. To be most effective, however, they must be employed near the initiation of smoking. The principal barrier to their successful implementation is the identification of nascent smokers. In order to identify these nascent smokers, clinicians traditionally have relied on self-report. However, the validity of self-report in adolescents is a hotly debated subject. To examine this subject more precisely, we compared self-report, serum cotinine, and DNA methylation values in a currently-being-collected cohort of high school students participating in a longitudinal examination of substance use initiation. Overall, 13% of high school sophomores report lifetime use of tobacco products with 1.5% (n=259) reporting use in the last week. In contrast, 9% of subjects (25 of 284) sophomores tested positive for serum cotinine, which indicates some use of tobacco/nicotine products over the past several days. Methylation at cg05575921, a recently developed epigenetic assay of cigarette consumption was not correlated with self-reports of lifetime or recent smoking (n=204). However, cg05557921 methylation, as assessed by Smoke Signature- a recently developed epigenetic assay, was highly correlated with intake serum cotinine values (Rsq =0.27; $p<0.0001$). We conclude that adolescents' self-reported smoking status is not reliable and, in fact, it markedly under-estimates their actual smoking. In contrast, the two objective markers of smoking status, serum cotinine and cg05575921, appear to be highly correlated. Because these two markers capture different aspects of cigarette consumption (acute vs. cumulative consumption), we suggest that examinations of adolescent smoking incorporate both of these measures to more accurately characterize smoking initiation and trajectories.