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On the challenges of hair testing to detect underreported substance use in research settings

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Hair testing; cannabis; alcohol

In a new manuscript published in *the American Journal of Drug and Alcohol Abuse*, Wade et al. examined the concordance between self-reported substance use and hair test results among a large sample of adolescents (1). This important study is among the first to compare hair test results to self-report in a large sample of adolescents (mean age = 11). Findings suggest that 10% of adolescents reported past-year psychoactive substance use and a mostly non-overlapping 10% tested positive for such substances.

We thank the authors for conducting this study and for properly acknowledging the various strengths and limitations of self-report and hair testing. In this commentary, we discuss some of this study's findings and expand upon some of the noted limitations. We further discuss two challenges to hair testing when used in epidemiology studies; the first is the ability of hair testing to detect two of the most common substances – alcohol and cannabis, the second is the feasibility of collecting analyzable hair samples.

Hair analysis and substance use detection capability

Compared to more commonly collected biospecimens such as urine, blood, and saliva, hair is unique in that it allows for many substances and their metabolites to be detected for many months post-exposure (2). However, there are some limitations to detection when using hair testing. An often-unacknowledged limitation is that exposure to psychoactive substances is typically not detectable within the first 1–2 weeks post-exposure. Therefore, while hair can allow us to detect exposure to substances within a wide window of time, it typically does not allow us to detect very recent use. Indeed, exposure to substances can typically be detected in urine, blood, and saliva within a few days post-exposure, but despite being able to detect

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current use, this is a much smaller detection window (3,4). As such, hair may arguably be among the best toxicological measures to indicate past-year substance use.

Another limitation of hair testing is varying sensitivity to detect exposure to different substances. Compared to urine testing, which is the most common method of biospecimen testing, hair testing appears to be superior in detecting exposure to substances such as cocaine and oxycodone (5). However, hair testing is less sensitive than other measures (e.g., urine testing) in detecting sporadic use of more common substances such as alcohol and cannabis (5,6). We stress this limitation because these were the two most prevalent substances in Wade et al.'s study. While hair test results from this study appeared to under-detect only about 2–3% of self-reported use of alcohol and cannabis, it is unknown to what extent detection was missed for use that was underreported (as drug use is commonly underreported in research). In contrast to the under-detection of reported use of opioids and stimulants being <1%, the under-detection of alcohol and cannabis was likely underestimated because hair testing is not the most efficacious in detecting lower frequency and lower volume use of these two substances (5–7).

While the authors did not appear to report the prevalence of adolescents self-reporting use *or* testing positive, 3% reported cannabis use and 6.1% of a largely non-overlapping sample tested positive, suggesting about 9% used cannabis based on self-report and toxicology results. This is lower than estimated prevalence of past-year cannabis use among 8th graders participating in the 2019 United States *Monitoring the Future (MTF) National Survey* (11.8%) (8). With respect to alcohol use, while 54% of adolescents in the sample reported having sipped alcohol, only 3% reported consuming one or more full drinks, and only 1.9% tested positive for alcohol exposure despite the range of concentrations being compatible with occasional consumption (9). Again, assuming largely non-overlapping prevalence as suggested by the authors, only about 5% are estimated to have engaged in consumption of full drinks. Yet, 19.3% of 8th graders in 2019 MTF were estimated to have consumed "more than just a few sips" of alcohol in the past year (and 6.6% were estimated to have gotten drunk) (8). It is unknown, however, how 8th graders interpret "more than just a few sips." Results are more in line with estimates of adolescents age 12–13 participating in the 2019 *National Survey of Drug Use and Health (NSDUH)* (10), in which 5.3% were estimated as having consumed a full alcoholic beverage that year (11). The true prevalence of use in this age group is likely somewhere *in between* NSDUH and MTF estimates as adolescents participating in NSDUH may underreport use given that a guardian is home with them during survey administration, and MTF, which is conducted in schools, may be subject to overreporting, in part, due to mischievous responding which is common among adolescents, particularly in school settings (12,13). Either way, these national prevalence estimates of students in the same age group may further suggest underreporting and/or under-detection of exposure via hair testing. We do believe, though, that potential under-detection would have been even more severe if the authors relied on urine-, blood-, or saliva-testing in this sample as such tests would typically only detect use within the few days prior to assessment. Ultimately, adding one of these tests with shorter detection windows to hair testing would certainly improve detection power (14).

In addition, it is not clear whether the analytical procedure used by the laboratory was sensitive enough to identify traces of exposure to a substance, namely a single intake (15). In such epidemiological studies, it might be convenient and more informative to set the limit of detection as the minimum criterion to establish use of a certain substance. The length of participant hair must also be congruent with the timeframe of interest. For example, very short hair that allows for (say) a four-month detection window will inevitably under-detect use that was closer to a year from assessment.

Hair analysis efficacy and feasibility of collecting and analyzing quality samples

In many respects, hair collection is more feasible than collection of blood or urine. Extensive training typically is not needed, there is less of a need for protective equipment and sanitary conditions, collection is not painful or invasive, storage and shipping is less burdensome, and hair can often be collected quickly in any type of environment (2,16). Hair testing is also efficacious in comparison to urine testing as it also allows us to avoid individuals attempting to dilute their sample or submit someone else's sample. We believe feasibility, however, can at times be offset by low response rates, usability of samples, and cost of analysis. We briefly discuss these three limitations below.

Relatively low hair collection response rates have been a limitation in many studies (17–19). Hair response rates tend to be lower than response rates for collection of saliva or urine (20), although some epidemiology studies that did not appear to require a hair submission have, in fact, had overall response rates as high as 91% (21). We commend the study staff overseeing the study published by Wade et al. for being able to achieve a 68% hair collection response rate in which only 4% refused. Low response rates are driven by refusal to provide hair, and also by insufficient hair samples (discussed below) (5,17). In one of our earlier street-intercept survey studies, only 33% of participants provided a hair sample (16). The main reasons for refusing were lack of interest (21.0%), not enough time (19.8%), not wanting someone touching their hair (17.7%), and not wanting their hair cut in public (13.8%). Of course, every study is different, but researchers should consider adjusting their methods to counter specific reasons for refusal. For example, we also provided an option for body hair collection, but this method is likely not as applicable or allowable regarding adolescent populations.

Despite low refusal rates, among responders in the study published by Wade et al., 23% had insufficient hair to provide. This relates to our second noted limitation to feasibility – insufficient hair quantity – as discovered during collection or later before analysis (17–20). It appears that sufficient hair quantity is more of an issue in studies that do not require hair collection because when hair collection is an inclusion criterion the researcher likely ensures beforehand there is enough hair to collect. We have never required hair submissions in our nightclub epidemiology studies as this would affect generalizability of substance use estimates, but we, too, have learned that when hair collection is optional, many hair samples we collect do not meet our 20 mg criterion. For example, in one of our recent street-intercept surveys outside of nightclubs, only 27% provided a hair sample, and of these, only 65%

met our criteria for analysis. Anecdotally, many of our staff members collected samples that were too small because participants demonstrated fear that too much would be cut or that the cut will lead to a visible bald spot. The quantity issue is particularly relevant when – as per the Wade et al. study – several classes of compounds are screened, and eventually, confirmed. In this scenario, multianalyte screening and confirmation methods are becoming promising opportunities for large cohort studies (22–24). It should also be noted that there are typically differential response rates and rates of usability of samples according to demographic characteristics (e.g., race, sex) and substance use (16,20,21). This may also require special attention in hair analysis studies as this can further bias results.

Finally, with respect to limitations to hair analysis feasibility is cost. While testing for rare substances (e. g., new psychoactive substances) can be expensive regardless of the type of biospecimen used, testing for exposure to common substances can typically be done using relatively cheap disposable urine- or saliva-testing devices, although only recent use can be detected. The cost for hair testing – even when only focusing on the most common psychoactive substances – can easily be over $100 per sample. The high cost of hair testing may be a reason why the authors of this study only analyzed a random sample of 6% of hair samples, focusing only on adolescents deemed low- or high-risk by an algorithm. Despite the respectable method of choosing which samples to analyze, however, the authors described a "surprising" finding that there was a higher-than-expected prevalence of substance detection (including cocaine use) among those deemed low risk. This is informative as it serves to remind us that, when possible, all available samples should be analyzed instead of a small subset (e.g., deemed to be high-risk). Although, it would likely be extremely expensive for the authors to analyze all of the >22,000 samples collected in their study. We have also limited some of our own toxicology analyses to detect unintentional synthetic cathinone ("bath salt") adulteration to subsamples of people reporting ecstasy use (25,26), but the surprising finding by Wade et al. reminds us that our results are likely biased as we did not analyze hair samples for those denying ecstasy use who might have actually used.

Conclusion

Given adequate hair length, hair testing can detect exposure to psychoactive substances within wide time-frames – much wider than with urine, blood, and saliva. However, hair testing cannot detect very recent exposure, and infrequent cannabis use in particular can be difficult to detect. Ultimately, a researcher's decision to use hair testing instead of other biological testing should depend on the type of population being studied. Hair testing appears to be much more efficacious when focusing on high-risk populations that report frequent substance use. But regardless of the study population and keeping both the strengths and limitations of hair testing in mind, the combination of self-report and biological testing has again proved effective to investigate trends and patterns of substance use.

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