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Letter to the Editor

Implementation of Mathematical Models to Predict New Cannabis Use by Urine Drug Testing: It Is Time to Move Forward

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To the Editor:

Cannabis (marijuana and hashish) constitutes the highest prevalence for psychotropic drug use worldwide, with an estimated 200 million users annually (1). As a result, urine drug testing (UDT) for cannabis use is very common. The testing is traditionally performed in a two-step process with an immunoassay for screening, followed by the confirmatory analysis of the 'presumptive positive' samples. Testing for cannabis is often part of workplace drug testing programs, doping analysis and roadside drug testing, where both immunoassay and confirmatory testing results are managed by trained professionals using working guidelines and validated procedures. In addition, point-of-care testing is performed in primary health-care settings, prisons, psychiatric wards and drug rehabilitation units (inpatient or outpatient) and sometimes in institutions where personnel in charge of UDT are less familiar with the fundamental principles and challenges of drug testing. Thus, due to the lack of certified training, standard procedures and documentation, interpretation of UDT results may become subjective and prone to error.

Correct postanalytical data interpretation in routine UDT for cannabis is crucial for an evidence-based and ethical approach toward the tested individuals, for whom a long period of multiple positive test results may occur. Bioaccumulation of lipophilic cannabinoids in the fat tissue of chronic users may in extreme cases cause detectable cannabinoids in urine for up to a month during abstinence (2). Positive UDT results in this period depend on the applied cutoff and urine dilution level. Without proper guidelines for interpretation of such data, the recurrence of positive results for longer time periods may in worst case lead to incorrect conclusion that the cannabis user has relapsed into new use. This may

in turn have negative consequences for the therapy, medical treatment and recovery of the patient. Misconceptions related to UDT for cannabis may be harmful and affect the mutual trust in the professional/patient or client relationship, which is already characterized by the clinician being placed in an authoritarian role. This is a particularly critical issue when a patient or a client in a drug rehabilitation program, struggling with withdrawal syndrome (3) during abstinence from cannabis use, is wrongly accused of a relapse. In prisons or while on probation, false interpretations of UDT results can have serious consequences with loss of privileges and incarceration. In other drug-testing scenarios, serious consequences may involve parenting matters and child custody, exclusion from treatment programs, loss of education/job and other penalties or punitive impacts.

Thus, major challenges are present when UDT for cannabis is performed in chronic, daily users enrolled in rehabilitation programs, where the rationale for testing is mainly to differentiate 'new use' from excretion of residual cannabinoids (originating from past use). In this article, we provide arguments for how mathematical models can be made available and applied to ensure correct evidence-based interpretation in cannabis testing to improve drug rehabilitation programs and clinical treatment.

A Mathematical Model for Cannabis Abstinence

For decades, researchers have sought to develop unambiguous mathematical models to detect new use in biological samples from cannabis users (4–7). Initial models were based on fixed acceptance criteria for the ratio of creatinine (CREA)-normalized concentrations

(between Urine Specimen 2/Urine Specimen 1) (4, 5, 8). These studies were refined and published in 2011 by Schwilke et al. in the most advanced mathematical model available to date to differentiate new cannabis use from residual urinary cannabinoid excretion among individuals with a history of daily cannabis use (9). The development and validation of the model was based on the analysis of >125,000 urine samples. We refer to the original publication for a detailed explanation; however, in short, the principle of the method defines an upper ratio limit above which new use is predicted at different probability levels (80%, 90%, 95% and 99%, respectively) using an exponential function with 'initial CREA-normalized concentrations of 11-nor-delta-9-carboxy-tetrahydrocannabinol' (eight intervals from 6 to 1,166 ng/mg) and 'time between specimen' (48–720 h) as variable parameters. Additionally, two specific rules must be applied in order to avoid false predictions, specifically in the initial phase (i) where urine concentrations may not have peaked after cessation of drug use, leading to a wrong prediction of new use from the two first specimens collected, and (ii) to account for prolonged elevation, which was observed in some users during the validation phase of the model, when the first day concentration was above 800 ng/mg (9).

The model by Schwilke et al. is acknowledged as a major advancement in the understanding of urinary excretion of cannabinoids in chronic users, and the publication has been cited in >40 peer-reviewed articles. Thus, it has been applied in research and clinical trials where use of the mathematical model has been the best tool to verify abstinence in study participants (10, 11). However, the model has not yet been implemented as warranted in routine UDT practice at the institutional level, where clinicians in charge of patient care and management act on the results. This situation is clearly a disadvantage and calls for action to encourage implementation of the knowledge gained from mathematical modeling into practical use.

Laboratory Data Are Necessary

To be able to implement a mathematical model practice in urine cannabis analysis interpretation, quantitative results must be available for the target urine metabolite 11-nor-delta-9-carboxy-tetrahydrocannabinol (THC-COOH), measured with confirmatory chromatographic and mass spectrometric methods, using the consensus cutoff value of 15 ng/mL for clinical UDT. This is the confirmatory cutoff appropriate for immunoassay cannabinoid screening at 50 ng/mL. It is recommended that CREA is determined for all UDT as it serves as a biomarker for dilution factor and evaluation of sample integrity. Thus, the CREA-normalized THC-COOH concentration (THC-COOH/CREA) can be calculated and reported by the laboratory. In the discussion and context here, it is implied that either gas chromatography or liquid chromatography coupled to mass spectrometry has been used for a specific quantitative determination of THC-COOH. Since immunoassays for cannabinoid screening are semiquantitative at best, they should not be used in cannabis prediction models developed from THC-COOH concentrations.

Clinical toxicology laboratories involved in UDT often analyze samples that have been pre-screened (on-site) with various immunoassays. Herein, we identify an obstacle, as 'presumptively positive' samples from point-of-care UDT may not always be forwarded for confirmatory, quantitative analysis, although guidelines have recommended this two-step strategy since the start of drug testing in the 1980s in the USA (12). The reasons for neglecting confirmatory testing can be patients/clients admitting a relapse of drug use or the clinician not being willing to wait for the confirmatory

result. It may also be due to lack of knowledge of the limitations of immunoassays (13, 14).

For drug testing personnel, in charge of acting on the final analytical result, even if THC-COOH/CREA results are available, knowledge of proper interpretation may be lacking. Previously, two different models were applied stating that if THC-COOH/CREA increased by 50% or 150%, respectively, it was indicative of new use (the 'ratio models') (4, 8). This may be true for a certain range of concentrations, but according to the model of Schwilke et al., this assumption cannot be applied to excretion of low, residual THC-COOH concentrations in chronic cannabis users (9). Consequently, different models must be applied for occasional and chronic users, respectively.

In summary, clinicians and others involved in the interpretation of cannabis UDT results are faced with a challenging task and for which access to support and expert guidance may be unavailable, leading to a risk of applying subjective rules of interpretation.

Misleading Advice from Vendors of Immunoassays

Another test regimen, which has negative consequences for proper interpretation, is the use of immunoassays at multiple cutoff levels in consecutive samples to monitor changes in drug use. In Denmark, this procedure has been widely advocated by vendors of point-of-care testing immunoassays (urine dipsticks). For example, it is recommended by vendors to screen for cannabis in urine samples at different cutoff values (e.g., 50, 200 and 300 ng/mL). However, this is a nonvalidated approach without scientific foundation due to the large variations in urine dilution, which may be larger than the ratios in cutoff factors. In fact, the urine CREA concentration is not measured when using this approach. According to the European Guideline for Workplace Drug Testing in Urine (15), in the description of integrity testing, urine samples are accepted for testing from 0.5 mM CREA and upward, in case the specific gravity is within acceptance criteria. Urine samples with CREA concentrations of 40 mM or higher are not unusual in a routine clinical biochemical laboratory. Thus, urine dilution may vary with a factor of 80. It is evident that a test regimen solely based on immunoassay testing with three cutoffs is associated with a higher risk of both 'false-negative' and 'false-positive' outcomes in its prediction of new use of cannabis. The spreading of such a misleading, pseudoscientific concept is harmful and may jeopardize proper use of UDT.

Point-of-care UDT testing by immunoassays can be justified as a rapid intervention tool—but only with necessary caution and knowledge about the pitfalls—and in the case of UDT for cannabis use, it is not possible to identify patients who have relapsed without a THC-COOH/CREA result measured precisely and accurately.

Urine analysis data from a chronic cannabis user are shown in Figure 1 to illustrate the potential shortcomings of immunoassay testing at different cutoff levels. Such THC-COOH/CREA curves have been used during interpretation of results for more than two decades (4), and it is included in the article to clarify the principle for new readers. It is obvious that immunoassay testing at different cutoffs, as discussed above, would have led to 'false-positive' prediction of new use at Days 38 and 49, where the urine CREA concentration increased by a factor of 3 and 4, respectively, compared with the previous sample. The model of Schwilke et al. predicted 'abstinent' at all probability levels between Days 19 and 25. For this chronic cannabis user, it took 54 days (after cessation of cannabis use at day 19) before a negative cannabis immunoassay screen (at 50 ng/mL) was
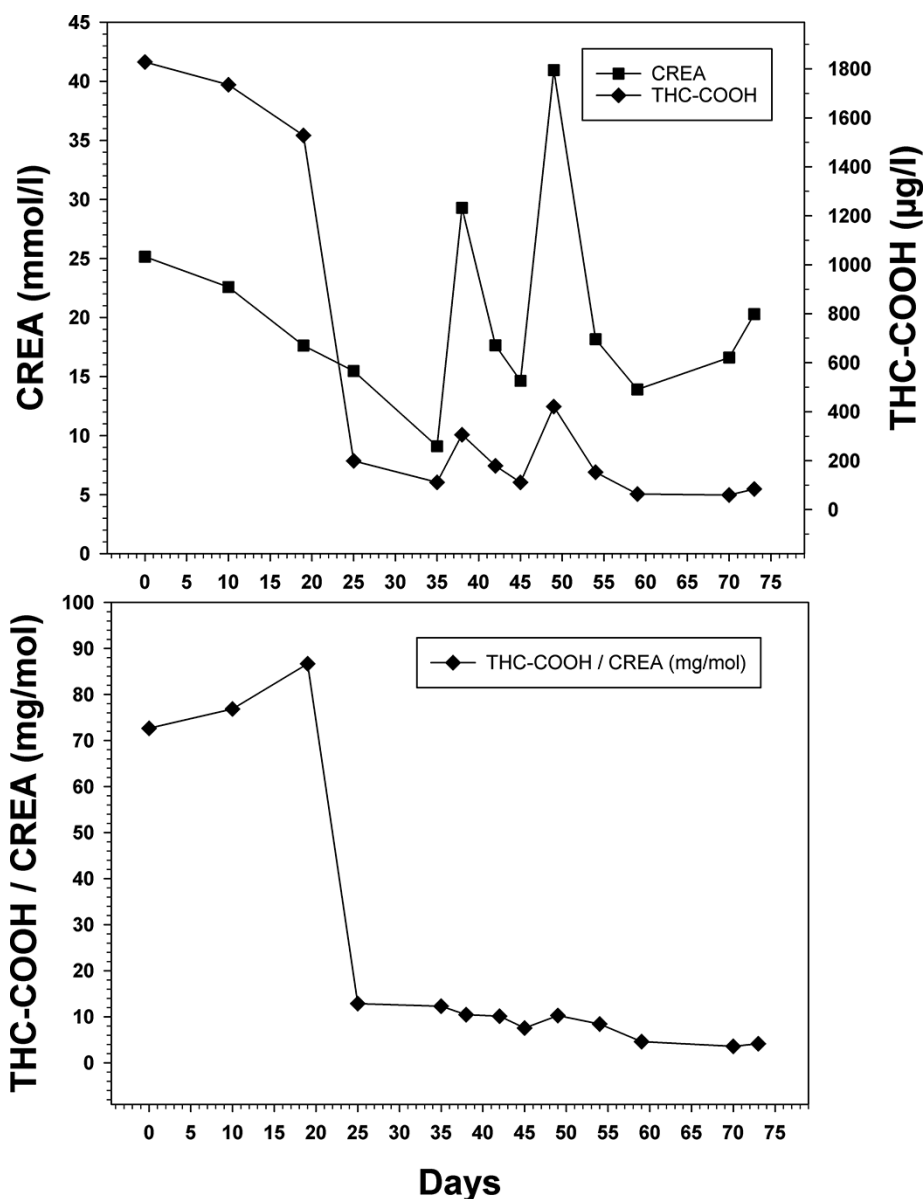
Figure 1. A graphical plot urine analysis data from a chronic cannabis user in drug rehabilitation treatment. The client was tested 13 times during a time period of 73 days. Abstinence was verified between Day 19 and 25 using the model by Schwilke et al. Top: CREA in mmol/L (units on left axis) and THC-COOH in μg/l (units on right axis). Bottom: THC-COOH/CREA in mg/mol. After Day 73, urine samples were screened negative by immunoassay testing (cutoff: 50 ng/mL), and confirmatory analysis of THC-COOH (cutoff: 15 ng/mL) was not performed.

obtained. As mentioned above, the model of Schwilke et al. can only be used to predict abstinence up to 30 days after cessation. Predictions from two specimens collected 54 days apart would be an extrapolation of the model. However, in the case shown in Figure 1, we interpret the continuous low THC-COOH/CREA values from Day 54 and onward as a prolongation of the abstinence period.

A Mathematical Model Online?

Since 2011, our drug testing laboratory has used an 'in-house' spreadsheet version of the mathematical model of Schwilke et al. (9). For almost a decade, we have offered analytical support based on calculations of this model for clinicians involved in cannabis UDT. However, we are reluctant to distribute this spreadsheet among

clinicians and other health professionals, due to the risk of incorrect use. This is mainly due to the 'two rules' mentioned above, which are essential for the accurate predictions, but which render the mathematical model more complex and require prior training and supervision. There is also a risk in utilizing a mathematical model's prediction of abstinence as a primary goal, although reduced drug use in chronic users (but not full abstinence) may be a criterion for successful treatment in an attempt toward recovery after a long history of drug use. In each individual case, interpretation tools should be used with caution and care (16).

From our experience with the application of the Schwilke et al. model in numerous, clinical cases, we advocate the introduction of a software version of the model. The program could be available on the Internet (free of charge), including validated

auto-interpretation/evaluation functions, graphical presentations of data and appropriate warnings if the testing regimen is not applicable (too long intervals between testing, outliers not fit for the model, etc.). Online access to the model could be linked with e-learning programs for education of registered users prior to full access. Theoretically, linking to real laboratory data would be preferred to avoid typing errors. However, a system sharing personal and sensitive data (stored in public health databases) with external sources may be difficult to establish and only work as a limited local solution. Instead, a cloud-based solution would be preferred without direct access to personal information.

Utility of the Schwilke et al. model depends on the frequency of testing. Some argue that the financial cost of urine testing limits its routine use and that clinicians should instead rely on self-reporting and structured interview strategies. However, the trend in application of high-performance liquid chromatography and tandem mass spectrometry for automated urine analysis, without the need for initial hydrolysis ([17], [18]), will probably lower the cost of clinical toxicology analysis ([19]). With access to an online evidence-based program to differentiate new use from residual excretion of cannabinoids, the utility of THC-COOH/CREA as an accurate and valuable biomarker could be improved.

Many countries in the world are now in the progress of legalizing and decriminalizing recreational cannabis use while simultaneously introducing new medical cannabis products on the market. In an era where cannabis users will find themselves in a gray zone between legalization, criminalization and medical treatment, drug testing scenarios for cannabinoids could be even more complex and confusing. In the European Union, data from 24 countries showed that an average of 50% of primary cannabis users entering treatment for the first time (in 2018), reported daily use of cannabis during the previous month ([20]). There has been a rising treatment demand from 2006 to 2016 ([20]) and in this time period cannabis resin and herbal cannabis increased in potency in both Europe and the USA ([21]). The negative health consequences associated with high-potency cannabis products are worrying. Therefore, the need for mathematical modeling of cannabinoids' levels quantified in biological matrices is more relevant than ever to achieve accurate and operational interpretations of UDT.

It is the authors' opinion that the risk of misinterpretations of data from UDT for cannabis—now and in future—is high. Thus, it seems to be the right time to introduce and implement online mathematical modeling in UDT for cannabis and CREA-normalized data in drug testing in general. This approach appears to have a unique strength, which may justify the costs. However, the use of UDT does not exclude the integrated use of patient/client self-reporting and drug testing. In fact, a panel of experts has concluded that multiple assessments in both self-reports and biological testing yield the most accurate drug use information, given that self-reports are collected independently of toxicology data ([22]).

Previously, in a discussion on moral and ethical issues of drug testing in patients with psychiatric disorders, we identified a need for clinical laboratory professionals to assume a more active and educational role and be involved in the interpretation of drug testing results ([23]). This is also relevant in UDT testing for cannabis, where the lack of consensus guidelines, persistent myths and misconceptions can influence the postanalytical use of UDT results. Clinicians may also be conflicted about their role in UDT, and it is crucial to establish a strong professional network and analytical support.

Limitations

There are several limitations of implementing mathematical models to monitor cannabis abstinence. One challenge is the distinction between occasional users and daily, chronic users of cannabis. These two groups show differences in pharmacokinetics. Consequently, the mathematical models predicting new use differ. The model by Schwilke et al. is applicable to daily, chronic users, but for occasional cannabis users, simple THC-COOH/CREA ratios must be used. Therefore, to use online algorithms for predictions, thorough knowledge of the drug use pattern of the individual patient is essential in order to characterize them within one of the two groups.

The model by Schwilke et al. uses THC-COOH/CREA values expressed in ng/mg. However, some countries use mmol/L as the unit for CREA in urine, and thus, THC-COOH/CREA may be reported in mg/mol. Major caution should be taken not to compare data with different units but to perform a simple conversion if necessary.

CREA-normalized data may be biased if individuals are using CREA supplements, increase their intake of meat or change their physical exercise levels. In this case, it may be justified to use specific gravity normalization ([24]).

There may be disagreement whether mathematical models are fully applicable for criminal proceedings and doping analysis, where sampling at certain time points may be difficult. Prison-based drug treatment programs may benefit from evidence-based interpretations of UDT for cannabis. It is not our objective to discuss the issue in detail here. However, we acknowledge that the applicability of mathematical models and online solutions (with operator input) may be limited to certain sectors in society, where drug rehabilitation scenarios seem to be the most promising.

We also have not discussed the use of THC-glucuronide measurements in urine to predict recent cannabis use ([25]), as determination of THC-glucuronide is usually not performed by routine UDT laboratories and as the criteria for using this model are strict.

Conclusion

Cannabis point-of-care UDT based solely on analysis with different cutoff levels are inadequate due to the wide range of urine dilutions observed, and there is a strong consensus that CREA-normalized concentrations of THC-COOH are essential. Although researchers are familiar with state-of-the-art mathematical models for interpretation of UDT for cannabis, these models are still unknown to many clinicians. It is time to move forward and implement mathematical models in order to benefit from urine cannabinoid testing and to support the many end users who need access to these tools on a regular, daily basis in support of existing rehabilitation program activities.

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Data Availability

There are no new data associated with this article.

References

1. United Nations (UN). *World Drug Report 2020*. United Nations Publication: Vienna, 2020. https://wdr.unodc.org/wdr2020/index.html (Accessed Feb 24, 2021).

2. Goodwin, R.S., Darwin, W.D., Chiang, C.N., Shih, M., Li, S.H., Huestis, M.A. (2008) Urinary elimination of 11-nor-9-carboxy-delta9-tetrahydrocannnabinol in cannabis users during continuously monitored abstinence. *Journal of Analytical Toxicology*, 32, 562–569.

3. Budney, A.J., Hughes, J.R. (2006) The cannabis withdrawal syndrome. *Current Opinion in Psychiatry*, 19, 233–238.

4. Huestis, M.A., Cone, E.J. (1998) Differentiating new marijuana use from residual drug excretion in occasional marijuana users. *Journal of Analytical Toxicology*, 22, 445–454.

5. Smith, M.L., Barnes, A.J., Huestis, M.A. (2009) Identifying new cannabis use with urine creatinine-normalized THCCOOH concentrations and time intervals between specimen collections. *Journal of Analytical Toxicology*, 33, 185–189.

6. Fraser, A.D., Worth, D. (1999) Urinary excretion profiles of 11-nor-9-carboxy-delta9-tetrahydrocannabinol: a delta9-THCCOOH to creatinine ratio study. *Journal of Analytical Toxicology*, 23, 531–534.

7. Karschner, E.L., Swortwood-Gates, M.J., Huestis, M.A. (2020) Identifying and quantifying cannabinoids in biological matrices in the medical and legal cannabis era. *Clinical Chemistry*, 66, 888–914.

8. Manno, J.E., Ferslew, K.E., Manno, B.R. Urine excretion patterns of cannabinoids and the clinical application of the EMIT-d.a.u. cannabinoid urine assay for substance abuse treatment. In: Agurell, S., Dewey, W.L., Willette, R.E. (eds.). *The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects*, 1st edition, Section 2. Academic Press: New York, NY, 1984; pp 281–290.

9. Schwilke, E.W., Gullberg, R.G., Darwin, W.D., Chiang, C.N., Cadet, J.L., Gorelick, D.A., et al. (2011) Differentiating new cannabis use from residual urinary cannabinoid excretion in chronic, daily cannabis users. *Addiction*, 106, 499–506.

10. Rabin, R.A., Kozak, K., Zakzanis, K.K., Remington, G., George, T.P. (2018) Effects of extended cannabis abstinence on clinical symptoms in cannabis dependent schizophrenia patients versus non-psychiatric controls. *Schizophrenia Research*, 194, 55–61.

11. Schuster, R.M., Gilman, J., Schoenfeld, D., Evenden, J., Hareli, M., Ulysse, C., et al. (2018) One month of cannabis abstinence in adolescents and young adults is associated with improved memory. *Journal of Clinical Psychiatry*, 79. https://pubmed.ncbi.nlm.nih.gov/30408351/

12. Phan, H.M., Yoshizuka, K., Murry, D.J., Perry, P.J. (2012) Drug testing in the workplace. *Pharmacotherapy*, 32, 649–656.

13. Reisfield, G.M., Bertholf, R., Barkin, R.L., Webb, F., Wilson, G. (2007) Urine drug test interpretation: what do physicians know? *Journal of Opioid Management*, 3, 80–86.

14. Reisfield, G.M., Salazar, E., Bertholf, R.L. (2007) Rational use and interpretation of urine drug testing in chronic opioid therapy. *Annals of Clinical and Laboratory Science*, 37, 301–314.

15. The European Workplace Drug Testing Society (EWDTS). *European Guidelines for Workplace Drug Testing in Urine*. EWDTS, 2015. http://www.ewdts.org/data/uploads/documents/ewdts-urine-guideline-2015-11-01-v2.0.pdf (Accessed Feb 24, 2021).

16. Baker, N.L., Gray, K.M., Sherman, B.J., Morella, K., Sahlem, G.L., Wagner, A.M., et al. (2018) Biological correlates of self-reported new and continued abstinence in cannabis cessation treatment clinical trials. *Drug and Alcohol Dependence*, 187, 270–277.

17. Andersson, M., Scheidweiler, K.B., Sempio, C., Barnes, A.J., Huestis, M.A. (2016) Simultaneous quantification of 11 cannabinoids and metabolites in human urine by liquid chromatography tandem mass spectrometry using WAX-S tips. *Analytical and Bioanalytical Chemistry*, 408, 6461–6471.

18. Hadener, M., Weinmann, W., van Staveren, D.R., Konig, S. (2017) Rapid quantification of free and glucuronidated THCCOOH in urine using coated well plates and LC-MS-MS analysis. *Bioanalysis*, 9, 485–496.

19. Kahl, K.W., Seither, J.Z., Reidy, L.J. (2019) LC-MS-MS vs ELISA: validation of a comprehensive urine toxicology screen by LC-MS-MS and a comparison of 100 forensic specimens. *Journal of Analytical Toxicology*, 43, 734–745.

20. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). *European Drug Report 2020: Trends and Developments*. Publications Office of the European Union: Luxembourg, 2020. https://www.emcdda.europa.eu/publications/edr/trends-developments/2020_en (Accessed Feb 24, 2021).

21. Chandra, S., Radwan, M.M., Majumdar, C.G., Church, J.C., Freeman, T.P., ElSohly, M.A. (2019) New trends in cannabis potency in USA and Europe during the last decade (2008–2017). *European Archives of Psychiatry and Clinical Neuroscience*, 269, 5–15.

22. Donovan, D.M., Bigelow, G.E., Brigham, G.S., Carroll, K.M., Cohen, A.J., Gardin, J.G., et al. (2012) Primary outcome indices in illicit drug dependence treatment research: systematic approach to selection and measurement of drug use end-points in clinical trials. *Addiction*, 107, 694–708.

23. Jensen, C.M., Breindahl, T. (2019) Patients in medical treatment for attention deficit/hyperactivity disorder (ADHD): are they at risk in drug screening? *ADHD Attention Deficit and Hyperactivity Disorders*, 11, 333–340.

24. Huestis, M.A., Blount, B.C., Milan, D.F., Newmeyer, M.N., Schroeder, J., Smith, M.L. (2019) Correlation of creatinine- and specific gravity-normalized free and glucuronidated urine cannabinoid concentrations following smoked, vaporized, and oral cannabis in frequent and occasional cannabis users. *Drug Testing and Analysis*, 11, 968–975.

25. Desrosiers, N.A., Lee, D., Concheiro-Guisan, M., Scheidweiler, K.B., Gorelick, D.A., Huestis, M.A. (2014) Urinary cannabinoid disposition in occasional and frequent smokers: is THC-glucuronide in sequential urine samples a marker of recent use in frequent smokers? *Clinical Chemistry*, 60, 361–372.