Impact of Lowering Confirmatory Test Cutoff Value in Pre-Enlistment Urine Cannabinoids Screening: About Five Years’ Experience in the French Gendarmerie

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The guidelines for screening of urinary cannabinoids require that all specimens testing positive should be confirmed by gas chromatography–mass spectrometry at a confirmatory test cutoff value of 15 ng/mL of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH). To assess the impact of lowering the confirmatory test cutoff value on the diagnostic sensitivity and efficiency of a cannabinoid testing program, the results of 986 confirmation analyses of positive screening tests, conducted in the framework of medical fitness examinations prior to enlistment in the French Gendarmerie between January 1, 2005, and December 31, 2009, were retrospectively studied. If the confirmatory test cutoff value of THCCOOH is set at 5 ng/mL instead of 15 ng/mL as recommended by guidelines, the number of confirmed results increases by 25.2%. The positive predictive value of the initial screening test rises from 63.9 to 80.0%. Only one true-positive applicant has appealed. His THCCOOH urinary concentration, which was incompatible with passive cannabis smoke exposure, was confirmed by another laboratory. The use of a confirmatory test cutoff value lower than that recommended significantly increases the diagnostic sensitivity of the screening program for urinary cannabinoids without altering its specificity.

Introduction

Like the whole of society, the armed forces are directly concerned by illicit drug use (1). This is why a systematic pre-enlistment urine testing program was implemented in 1990 by the French Gendarmerie (police force with military status) (2).

Based on the same principles as workplace testing, a typical pre-enlistment drug testing program consists of an initial screening immunoassay test for cannabis. If the immunoassay is positive, confirmatory testing using gas chromatography–mass spectrometry is required to definitively establish the identity of the drug or drug metabolite and report the specimen as positive. Cutoff concentrations are used to determine whether the screening and the confirmatory results are positive or negative. Screening and confirmation cutoff levels are set for each tested substance according to several parameters, including pharmacokinetics (particularly metabolism and excretion, which are responsible for the detection window in biological matrices), passive exposure (e.g., passive cannabis smoke exposure) and food or licit drug interactions (e.g., for opiates). Cutoff values usually comply with guideline values set by the U.S. Substance Abuse and Mental Health Services Administration (SAMHSA) or by an expert group like the European Workplace Drug Testing Society (EWDTS) (3, 4).

With 12.4 million people admitting having used it more than once in their lifetime, cannabis is the most popular illicit substance in France (5). Its use predominantly involves young adults. In 2005, 47.5% of people aged 18 to 25 used cannabis at least once in their lifetime and 8.7% are regular users (5). As confirmed by recent epidemiological studies conducted within the French Army and the French Navy, the use of cannabis is a major issue for the armed forces, because the 18- to 25-age range is the target population for recruitment and the major part of the military strength (1). The urinary analyte that characterizes exposure to cannabis is 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH). THCCOOH is the main metabolite of the psychoactive substance Δ9-tetrahydrocannabinol (THC). In 1994, due to the improvement in the specificity of the antibodies directed against THCCOOH used for the immunoassay test, the initial screening test cutoff for cannabis was lowered from 100 to 50 ng/mL. This resulted in a rise in the diagnostic sensitivity of the tests (number of true positives increasing from 23 to 53%) without reducing specificity significantly (the loss of specificity is below 3%) (6, 7).

The screening test cutoff of 50 ng/mL seems to be adequate to detect the use of cannabis on the basis of both regular and recent occasional use. Nevertheless, some authors have demonstrated that a screening test cutoff of 25 or even 20 ng/mL THCCOOH would improve the diagnostic sensitivity of the test while preserving its specificity (8, 9). Established in 1988, the SAMHSA urinary confirmatory test cutoff of 15 ng/mL THCCOOH was not modified when the screening test cutoff was lowered from 100 to 50 ng/mL. This confirmatory test cutoff value continued to correlate with results provided by immunoassays in use during that period (10). Today, this confirmatory cutoff appears to be very high compared to the detection limits of GC–MS techniques currently used for the confirmation test.

The possibility of setting a lower confirmatory test cutoff is rarely mentioned in the scientific literature. Studies on drug testing programs primarily concern the performance characteristics of the different screening immunoassay tests that have been developed and marketed. They essentially determine the sensitivity and specificity of the screening tests compared to the results of confirmatory testing using the recommended confirmatory cutoff. In these controlled studies, screening tests are performed by operators with strong laboratory experience and the samples are from drug-added specimens or donor urine specimens at specified concentrations. In general, these studies only include a few cases. Theses study designs do not represent what can be observed in pre-enlistment or workplace medical fitness examinations.

Applicants for enlistment in the French Gendarmerie undergo a medical examination, including a point-of-collection screening immunoassay for urinary cannabinoids. The Forensic Science Institute of the French Gendarmerie (IRCGN) performs all confirmatory testing of screening tests conducted
at the national level by medical centers of the French Gendarmerie.

The confirmatory test cutoff value used by this laboratory is set at 5 ng/mL instead of the recommended 15 ng/mL. To assess the impact of lowering the confirmatory test cutoff value on the diagnostic sensitivity and efficiency of a cannabis testing program routinely involving many cases, the results of confirmation analyses, conducted in the framework of medical fitness examinations prior to enlistment in the French Gendarmerie between January 1, 2005, and December 31, 2009, were retrospectively studied.

Materials and Methods

Urine collection and on-site analysis

The device used during the study period in the medical centers of the French Gendarmerie is the monoparametric Syva RapidTest Cartridge (THC) (Dade Behring, Paris, France). The cutoff defined by the supplier of this test is 50 ng/mL. All positive urine specimens are sent to IRCGN for confirmation testing by GC–MS (2). Urine specimens that tested positive were aliquoted by paramedical personnel into two tamper-proof 30 mL plastic bottles. They were both identified by means of an adhesive label with the following data: anonymity number, year of collection, identification of the medical center in which the screening test was performed, signature of both the patient and the paramedical personnel who supervised the collection. The samples are usually transported by post at ambient temperature. On arrival at the authors’ laboratory, the samples are stored at −20°C. Only one sample is analyzed; the second is kept in the freezer in the event of disputed results and subsequent need for a second assessment.

The samples and results considered in this study were obtained exclusively through the routine medical examinations conducted in accordance with medical fitness standards in the French Gendarmerie. In this context, applicants are previously and regularly informed that “drug use, detected by the necessary diagnostic tests, is a general cause of unfitness for service” (2). The applicants sign a statement on the chain of custody form, certifying that the specimen identified on the form was necessary diagnostic tests, is a general cause of unfitness for service"

informed consent for the work to be undertaken.

Standards and reagents

All reagents are of analytical grade. Methanol, hexane, and ethyl acetate are purchased from Sigma Aldrich (Saint Quentin Fallavier, France). Concentrated ammonium hydroxide and glacial acetic acid are from Fisher Scientific (Illkirch, France). The enzymatic hydrolysis is performed by beta-glucuronidase from Escherichia coli 400 UI/L (APOH Technologies, Villeneuve Saint Georges, France). The derivatizing agent is bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA) (Alltech, Templemars, France). Ultrapure type I water is produced through a Millipore gradient A10 system with Q-Gard I and Quantum EX cartridges and filtered on a Millipore 0.22 µm Millipack filter (Millipore, Molsheim, France).

Calibrators and controls

The Cerilant reference solutions, namely THCCOOH and trideuterated 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH-d3), each at the concentration of 0.1 g/L in methanol, are purchased from LGC Standards (Molsheim, France). These stock solutions are diluted with methanol to obtain a THCCOOH solution at the concentration of 5 mg/L and THCCOOH-d3 at the concentration of 1 mg/L. The quality control material is Liquichek urine toxicology control from Bio-Rad (Marne la Coquette, France). The target value for the free THCCOOH is 18.5 ng/mL.

Calibrators and specimen preparation

The calibration samples are prepared in duplicate from 2 mL drug-free urine by spiking THCCOOH at 40 and 100 ng/mL with the nondeuterated THCCOOH solution. A run comprises the calibration samples, a negative urine, a quality control and applicant samples. The method described in the following is able to quantify total THCCOOH (i.e., both conjugated and free THCCOOH). Therefore, a preliminary enzymatic hydrolysis is needed. The urine sample of 2 mL is adjusted to a pH between 6.5 and 7.0 by adding diluted ammonium hydroxide or acetic acid. Then, 50 µL of beta-glucuronidase are added. The tube is left for at least 14 h at 37°C. After the hydrolysis, 20 µL of the deuterated internal standard and 200 µL of 10% acetic acid (in water) are added. The liquid–liquid extraction is performed by adding 5 mL hexane–ethyl acetate (90/10, v/v). After gentle shaking, the tubes are centrifuged 5 min at 1,780 × g. The upper organic layer is transferred to a second borosilicated 5 mL tube and subsequently evaporated at 40°C under a gentle stream of filtered air. Forty microliters of the derivatization reagent (BSTFA + 1% TMCS) are then added to the tube and left for 20 min at 80°C. One microliter of this preparation is analyzed by GC–MS.

GC–MS analysis

Analysis and quantitation are performed by GC–MS Shimadzu GC 17A and QP5000 (Shimadzu, Champs sur Marne, France) using a chromatographic column from Agilent Technologies: J&W HP-1MS Ultra Inert capillary 12 m × 0.20 mm × 0.33 µm (Interchim, Montlucon, France). The software is GCMSsolution (Shimadzu). The injection is made in splitless mode at 280°C, with a constant flow of helium at 0.9 mL/min. The temperature program as follows: 90°C for 4.5 min; from 90°C to 127.5°C at 37.5°C/min; from 127.5°C to 210°C at 13.5°C/min; from 210°C to 250°C at 5°C/min; from 250°C to 320°C at 25°C/min; 320°C maintained for 4 min. The electron impact mass spectra are acquired in the selective ion monitoring mode (SIM). The electron beam voltage is 70 eV. The ions for the analyte and for deuterated internal standard are monitored (quantification ions in bold): THCCOOH m/z 473, 371, 488 and THCCOOH-d3 m/z 476, 374, 491.

Validation

The response function is a linear unweighted function (y = ax). The method validation was based on the total error concept (11, 12) and performed using e-noval software (Chemcad, Obernay, France). Validation standards at four concentrations (3, 10, 50 and 100 ng/mL) were analyzed with independent calibration curves on each of three series (intermediate precision) of four replicates (repeatability). The
The cutoff value used by the authors’ laboratory is set at 5 ng/mL. The confirmatory test accredited according to the ISO 17025 standard by the French Accreditation Committee (COFRAC). The confirmatory test cutoff value used by the authors’ laboratory is set at 5 ng/mL instead of the recommended 15 ng/mL.

Data analysis

In a retrospective study like the one presented in this paper, not all performance parameters of the urine screening test can be calculated, particularly diagnostic sensitivity and specificity. Because only the urine specimens for which the screening test proved positive are sent to the laboratory for confirmation purposes, no information is available about the negative screening tests. The positive predictive value (PPV) is the only parameter that can be calculated. In the hypothesis that all specimens sent to the lab had a positive screening test, the PPV corresponds to the percentage of confirmed samples.

Results

During the study period, 62,135 medical fitness examinations involving urine screening for cannabis use were conducted prior to enlistment. Of these, 1,004 had positive screening results, requiring subsequent confirmation testing at the IRCGN toxicology department. Among the 1,004 samples received, only 986 have been analyzed. The remaining 18 specimens had urine volumes less than 2 mL. For the majority of these samples, the bottle had not been sealed properly and contents emptied during transport.

Number of confirmations and performance of screening test

In the study period, 789 urine tests (80.0%) were confirmed at a THCCOOH concentration equal to or over 5 ng/mL; 197 samples did not confirm (absence of THCCOOH or concentration under the cutoff of 5 ng/mL) (Figure 2). Among the 789 samples confirmed, only one applicant has appealed. His THCCOOH urinary concentration (>15 ng/mL), which was incompatible with any passive cannabis smoke exposure (13, 14), was confirmed by another laboratory (Institute of Aerospace Medicine of the French Military Health Service).

Distribution of urinary THCCOOH concentrations and influence of the confirmatory test cutoff

The distribution of THCCOOH concentrations in GC–MS confirmed urine samples for the entire study period is shown in Figure 2. During the study period, 55.5% of urine samples subjected to confirmation analysis showed a concentration lower than the initial screening test cutoff (50 ng/mL) and 20.2% of confirmed samples had a THCCOOH concentration value in the concentration range 5 to 15 ng/mL.

Table 1 presents the distribution in number of samples confirmed when different confirmatory test cutoffs are applied a posteriori. Using a confirmatory test cutoff of 10 ng/mL instead of the recommended cutoff of 15 ng/mL raises the number of true positives (samples confirmed) by 12.5%. The PPV comes to 71.9% instead of 63.9%. When the confirmatory test cutoff used in the authors’ laboratory (5 ng/mL) is applied, the number of true positives rises by 25.2%, and the PPV is 80%. Using a cutoff higher than 15 ng/mL induces a dramatic fall in the number of confirmed samples and decreases the PPV below 60%.

Discussion

Syva RapidTest, like other point-of-collection urinary cannabinoid screening immunoassays, will yield a very small number of false positives and presents a PPV higher than 90% for a confirmatory test cutoff of 15 ng/mL (15–18). Although a confirmatory test cutoff of 5 ng/mL was applied, the PPV obtained in this study is lower than the values reported in the scientific literature. This illustrates the differences in performance between a study under standardized conditions and a study in real conditions of use. These unexpectedly low PPV values probably result from an excess of false positives combined with a lack of true positives in the use of routine tests. False positives can result from a misreading of the screening test by uninitiated personnel. Indeed, these tests require an inverse reading (the presence of the colored band indicates a negative result). Moreover, when confronted with a doubtful screening result, many medical practitioners prescribe a confirmation analysis, which contributes to an excess of false positives. Furthermore, the specificity of screening immunoassays is limited due to cross-reactions of the anti-THCCOOH antibodies with molecules like nonsteroidal anti-inflammatory drugs (NSAIDs) (19) and, to a lesser extent, proton pump inhibitors (Efavirenz) (15). Concerning the deficit of true positives, this could result from the failure to request confirmatory testing.
when the positive screening test is reinforced by the confession of the applicant during the medical examination.

Assays performed in the 789 urine samples for which the presence of THCCOOH was confirmed show a large dispersion of concentrations. More than half (55.5%) of urine samples in which the presence of THCCOOH was confirmed had a lower confirmatory concentration than the initial screening test cutoff of 50 ng/mL, and samples with THCCOOH concentrations less than 15 ng/mL represented 20.2% of confirmed analyses. THCCOOH concentrations far below the screening test cutoff are commonly observed (8). This phenomenon is due to cross-reactions between the many metabolites of different cannabinoids present in marijuana smoke, which contribute to the screening positivity rates (20). Moreover, cold chain failures when transporting specimens to the laboratory generate a decrease in THCCOOH sample concentration between screening and confirmatory testing and thus contribute to the measurement of lower confirmatory concentrations. According to Skopp et al., there is a 13.4% decrease in THCCOOH when the urine sample is stored at room temperature (20°C) for 48 h. There is a 21.2% THCCOOH loss when the sample remains at room temperature for 120 h. Mechanisms of decarboxylation and bacterial or fungal degradation of THCCOOH predominantly account for this decrease in THCCOOH concentration, which also contributes to the increase in the number of false positives (21).

Distribution of confirmatory concentrations below the recommended confirmatory test cutoff value of 15 ng/mL in urine samples, which tested positive with an initial screening cutoff of 50 ng/mL, is an argument that supports the use of lower confirmatory test cutoff values. In this study, the cutoff of 5 ng/mL increases the number of true positives by 25.2%. Few publications report the criteria for setting the confirmatory test cutoff of THCCOOH in cannabis testing programs. In a study using the 20 ng/mL initial screening test cutoff, Wingert et al. recorded a 7.3% increase in the number of true positives when reducing the confirmatory test cutoff from 10 to 5 ng/mL (9). THCCOOH can be detected in urine from the first micturition after cannabis use in the case of an acute exposure. Nevertheless, when analyses are performed on the first urine voided after cannabis smoking, Huestis et al. demonstrated that only half of the subjects who have smoked a cigarette containing 15.8 mg of THC, and only 83% of subjects who have smoked a cigarette containing 33.8 mg of THC, present a urinary THCCOOH concentration above the cutoff of 15 ng/mL (22). The use of a lower confirmatory test cutoff can also effectively reduce the number of falsely unconfirmed screening in urine specimens adulterated by dilution (whether with the intention to suborn the test or as a result of ingesting large amounts of liquid in the hours before collection) as well as the decrease in urinary THCCOOH during the transfer of samples (23, 24). Some authors recommend using no confirmatory test cutoff at all, instead identifying THCCOOH by its mass spectrum only (based on the mass pattern identification criteria) (8). This approach makes it necessary to define the analytical performance of the method, notably the LOD. This prerequisite is critical when several laboratories perform confirmation analyses within the same drug testing program.

The possibility of passive inhalation of cannabis smoke is an argument often suggested against lowering the confirmatory test cutoff value. Subjects have indeed sometimes used the concept of passive inhalation of cannabis smoke to justify positive tests. Available studies on THCCOOH detection in urine after passive exposure are related to acute exposure and experimental conditions largely more drastic than in real-life situations (confined space, no ventilation, large number of
smokers and very high THC level of the cigarette) (25, 26). If THCCOOH is detected by GC–MS in urine after passive exposure, these experiments establish that despite drastic exposure conditions to sidestream cannabis smoke, the immunoassay screening test results are still negative at a cutoff value of 50 ng/mL (13, 14).

Consequently, it seems very unlikely that passive exposure to cannabis smoke should solely account for a positive urine screening test. Even if the urine sample of an applicant who has really been passively exposed to cannabis smoke would appropriately be subjected to confirmatory testing (due to a doubtful screening test, a misreading or a cross-reaction, for example), these studies also show that THCCOOH concentrations higher than the 5 ng/mL confirmatory test cutoff value can only be measured within six hours after exposure (13, 14).

This window of detection should be in reality relatively short, because these data were derived under particularly extreme experimental conditions of passive cannabis smoke exposure. In addition, medical fitness standards provide advanced information to applicants before the drug screening test and medical examination take place during working hours and away from an eventual passive exposure. Moreover, experience accumulated by the authors’ laboratory has demonstrated that use of a confirmatory test cutoff of 5 ng/mL did not generate an excessive number of disputed results and appeals. Only one appeal was recorded during the five-year study period, for which the results were confirmed by another laboratory and for which THCCOOH urinary concentration was incompatible with any passive cannabis smoke exposure (>15 ng/mL). This implicitly reflects the acceptance of confirmatory testing results by the applicants and attests the reality of their cannabis use.

Conclusion
The retrospective study of 986 urine confirmation testing results over five years has examined the influence of the urinary cannabinoid confirmatory test cutoff in pre-enlistment drug testing programs, such as the one set up within the French Gendarmerie. The choice of a confirmatory test cutoff of 5 ng/mL THCCOOH over the recommended cutoff of 15 ng/mL considerably increases the PPV of initial screening tests and therefore increases the diagnostic sensitivity of the cannabinoids screening program as a whole. Choosing a lower confirmatory test cutoff may compensate for sample adulteration by dilution or degradation of the analyte during transportation to the laboratory. Mass spectrometry easily ensures the analytical specificity of confirmatory testing at this level of concentration. In addition, recent studies have confirmed that passive exposure to cannabis smoke cannot solely generate positive results in initial screening tests using the recommended screening test cutoff of 50 ng/mL THCCOOH. Finally, if detection of THCCOOH in the urine of an individual proves cannabis use, the determination of the confirmatory test cutoff value cannot aim to differentiate between occasional and chronic users, to diagnose a problematic consumption (harmful use and dependence) and to determine the medical fitness to hold some employment. Assessment of addictive behaviors should primarily be based on interview and clinical data collected during medical fitness examinations, and may involve questionnaires and validated scales such as the cannabis abuse screening test (CAST) (1).

References


