# In new era, cannabis testing a mixed bag

## Anne Paxton

**January 2017—**Extended cruises down the rivers of Europe and life without alarm clocks might figure in a vision of retirement for some people, but don't include toxicology expert Marilyn A. Huestis, PhD, in that contingent, at least for now.

After 23 years with the National Institute on Drug Abuse, she retired last February from her position as senior investigator and chief of chemistry and drug metabolism. However, she is involved in dozens of research protocols, she continues to teach, and the phone has hardly stopped ringing with calls from U.S. regulatory agencies, state legislators, foreign governments, and diagnostics manufacturers seeking her services as a consultant. In fact, she says, "I'm working twice as much."

Much of the demand for her expertise can be traced to the dramatically changed landscape for legal use of marijuana.

Ten years ago, when California passed the nation's first law permitting patients to use medical marijuana, bookmakers would have given long odds to the chance of many other states doing likewise, never mind decriminalizing recreational use. And today, marijuana remains a substance that the federal government still classifies, alongside heroin and LSD, as a Schedule I drug. But going in to the November 2016 election, four states and the District of Columbia had already legalized recreational use of cannabis and 25 had legalized medical marijuana. Post-election, those numbers are eight (plus DC) and 29 (plus DC).

The trend is unmistakable, although opinions on its desirability differ. Dr. Huestis, who has helped Canada with legalization issues and serves as senior scientific advisor to NMS Labs and as a consultant to the U.S. Department of Transportation, is concerned about the long-term impact of wider access to marijuana, already the most popular recreational drug in the U.S. But one thing people can agree on is that the eased legal climate for marijuana is changing the methods and usage patterns of cannabis testing in the laboratory, at the point of care, in workplaces, and at the roadside.

Cannabis testing can provide evidence of criminal drug offenses and employee drug use, be an indicator of possible impaired driving, monitor adherence to substance abuse treatment or civil orders or compliance with its use as a therapeutic drug, or provide clinical data in emergency care. To meet the demand, "there are new point-of-care technologies and devices, and laboratories continue to expand the list of analytes they measure in their cannabinoid assays," says Paul J. Jannetto, PhD, director of the clinical and forensic toxicology laboratory at Mayo Clinic, Rochester, Minn.

But with at least four biologic matrices, and minimally trained personnel sometimes handling specimen collection, sources of preanalytic error abound, and questions surround interpretations of results, whether they are positive or negative. Although sensitivity and specificity of testing have improved, "Cannabis is a very complex drug that contains over a hundred cannabinoids, and it's quite a challenge from the laboratory standpoint, not to mention the implications for legal testing and judging impairment," Dr. Jannetto says.

Most current laboratory test menus offer an immunoassay screen using urine, with mass spectrometry (LC-MS or LC-MS/MS or GC-MS) performed as a confirmatory test. These tests are typically looking for 11-nor-9-Carboxy-Δ9-tetrahydrocannabinol (THC-COOH), the main inactive cannabis metabolite, while the increasingly popular oral fluid devices used for roadside testing are calibrated for Δ9-tetrahydrocannabinol (THC), the main psychoactive parent drug, Dr. Jannetto notes.

But given the new and emerging regulatory frameworks for cannabis, laboratories can expect the traditional testing practices to change, Dr. Jannetto and Dr. Huestis agree. In his frequent presentations before medical

audiences, Dr. Jannetto finds clinicians and laboratorians generally are seeking more information on when and how they should test and what analytes they should be looking for, especially in medical formulations.

While the biologic matrices of cannabis testing include blood, urine, oral fluid, and hair, most testing done now is performed on urine, Dr. Jannetto says. “Unfortunately, that means most of the assays are looking for the inactive metabolite THC-COOH rather than the psychoactive components of cannabis, and THC-COOH could be an indicator of past use, possibly even several days prior to the collection of the urine sample,” Dr. Jannetto says. “So answering the most frequent questions—did the person recently ingest marijuana, and was the person impaired at a certain point in time?—becomes difficult.”

The correlation between impairment and blood levels of the psychoactive ingredient of marijuana, THC, is not settled, and differing laws set standards across the world as well as within the United States, Dr. Jannetto says. In some states (Georgia, for example), any concentration of THC or THC-COOH is considered impairment. Washington state, one of the first to authorize recreational marijuana use in 2012—has “per se” or DUID (driving under the influence of drugs) laws. “In this state, they have a 5 ng/mL cutoff, and if you’re higher, then you are automatically considered impaired,” he says.

Dr. Huestis believes a cutoff of 5 ng/mL is far too high. “That’s because your occasional cannabis user—anybody who uses it less than daily—will be dropping below 5 ng/mL in about 2½ hours. So they are still impaired for driving, yet they would be under the limit.” The chronic users show a different pattern, she notes. “In our study, all of our chronic, frequent cannabis smokers were below 5 ng/mL by 19 hours. They were heavy users smoking on average 10 joints a day for more than 10 years.” An Australian study, she adds, reported one person who was above 5 ng/mL for 129 hours. “So you can see how complex it is setting a number.”

“Obviously, testing for alcohol is similar to what we’re trying to do with cannabis,” Dr. Jannetto says. “You have concentrations in samples, you’re using blood, serum, or oral fluid, and just as with alcohol, there are legal deterrents set up so that you would not drive or go to a workplace under the influence. However, one of the key differences is there’s currently no really good way to back-extrapolate to verify THC levels at the time they say someone was being pulled over or after a collision.” In addition, a single set concentration of THC that is directly linked to impairment hasn’t been defined for all users, he says.

**Defining impairment has proved** to be tricky. A May 2016 study sponsored by the AAA Foundation for Traffic Safety (“An Evaluation of Data from Drivers Arrested for Driving Under the Influence in Relation to Per se Limits for Cannabis”) found little relationship between impairment as judged by police officers and drivers’ blood concentration levels of THC, Dr. Jannetto says. “And that obviously brings up a whole host of legal issues, especially when you start talking about those zero-tolerance laws based on THC-COOH. THC-COOH doesn’t mean impairment at all.”

Especially with blood serum testing, the half-life of the psychoactive component of THC is actually very short, he says. “So if you think someone is impaired and you wait even two hours, by that time the blood serum concentration has decreased.” In addition, concentrations in urine don’t correlate back to dosage.

**Several states have written into** their laws that they can take saliva samples, although in the U.S., additional legislation is often needed to authorize use of saliva in traffic stops. Oral fluid testing, though, is already common in Australia and other countries where, unlike the U.S., random traffic stops are authorized and routine, Dr. Huestis says. “If an oral fluid test is positive, in Australia they will take an evidentiary oral fluid sample with a different device. In Germany, if the oral fluid sample is positive, they will perform a blood draw.”

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Marilyn Huestis, PhD

Time of collection of specimens is pivotal—and problematic. For example, in a recent study, Dr. Huestis and colleagues tested individuals’ THC concentrations at intervals after the initial test. “The concentrations in the blood that caused impairment while they were driving, considered equivalent to .05 percent alcohol, which most of the world uses, or .08 percent in the U.S. and England, those concentrations drop 73 percent within 30 minutes and 90 percent in 1.4 hours.” In the U.S., she points out, it takes an average of 1.4 to four hours to collect a blood sample, either after a traffic stop or after a crash.

All states and Washington, DC, set .08 percent blood alcohol concentration as the point at which a driver may be charged with driving under the influence or driving while impaired. For commercial drivers, the set point is .04 percent. How should limits be set for THC that indicate a comparable level of impairment? That’s the giant question that has yet to be answered, says Dr. Huestis. “Every state legislator I’ve talked to wants one number that will say everybody is impaired.”

Even alcohol doesn’t have such a number. Not only are there significant differences between the U.S. and England (.08 percent) and Europe (.05 percent), but the Scandinavian countries go even further, setting a limit of .02 percent. In Sweden, Denmark, Finland, and Norway, she says, “when people go out for dinner and drink wine, everyone takes a taxi home,” or public transit, which is much better as a rule than in the U.S. “Nobody thinks about driving home, even with one or two glasses of wine.”

Generally, impairment is not strictly correlated with blood concentration of any substance, Dr. Huestis says. “If you are alcoholic, you might be much less impaired at .08 percent than an occasional drinker, but we don’t take that into consideration. We say it’s ‘per se’ illegal to drive with a .08 percent alcohol. You don’t have to prove they’re impaired; it’s what society sets as the limit.”

Alcohol and cannabis have different manifestations in the body, making the presence of THC more difficult to interpret, Dr. Huestis points out. “Unlike alcohol, THC is very lipophilic; it loves fatty tissues. When anybody smokes a cigarette, whether it’s tobacco or cannabis, the drug gets distributed throughout the body and slowly leaches out. If you’re an occasional smoker, the amount leaching out is very low, it’s not really detectable, and it probably doesn’t have much effect. But if you are a chronic, frequent smoker and you build up huge body burdens of THC, the active compound that is stored is what is active in the brain. And the brain, with all the myelin it contains, is one of the fattiest tissue organs we have.”

Even after 30 days, Dr. Huestis’ study showed, chronic, frequent cannabis smokers had measurable low THC concentrations (0.3 ng/mL) in their blood. Her team did PET imaging in that group, finding that chronic, frequent

cannabis smokers had significant down-regulation of the CB1 cannabinoid receptors in the brain, the endogenous cannabinoid system.

This neurotransmission system "was not developed so people could smoke cannabis and get high," she says drily. "It has critical functions with memory, with reproduction, with movement, and many other important functions. And basically, when people use cannabis, they hijack that function. When people use a lot of cannabis, the body tries to counteract that and it reduces the number of cannabinoid receptors that the THC can stimulate."

Her research team at NIDA found that the density of receptors, after subjects had no access to drugs for 30 days, returned to normal. "There was no significant difference between the chronic, frequent smokers' density of cannabinoid receptors in their brain and healthy controls' density. But when we did testing of psychomotor performance, at one, two, or three weeks of no cannabis use, we were really surprised to see that while there was some improvement, there was significant psychomotor impairment, even more than three weeks after the last use."

Users of cannabis do develop tolerance in some respects, Dr. Huestis notes. "But you don't get 100 percent tolerance in any effect, and you don't get any tolerance in some effects. So there's never complete tolerance. For instance, we have beautiful diagrams showing if you ask both occasional and chronic users about subjective effects (how high do you feel now, how stoned are you?), the occasional users will show greater response and at much lower THC concentrations. The chronic, frequent users may get up to the same level of subjective response, but they have to have lots higher THC concentrations to get there."

So there is a pressing need, from her standpoint, to find a marker of recent cannabis use. In research she conducted, "we found if you can measure cannabinol [CBN] and some other metabolites of THC, then—whether you are an occasional or a chronic, frequent user—all have short detection times, shorter than the windows of impairment."

In another study, her team identified one of the 109 cannabinoids in the marijuana plant called cannabigerol, or CBG. "That gave us the best detectability. So we have some markers that indicate recent use, but they are inclusionary, not exclusionary." She knows this because she has dosed subjects in the study herself and measured cannabinoid concentrations, and in some people, especially occasional users, she couldn't find those compounds. "So if you find it, it means recent use. If you don't find it, it doesn't mean you can exclude recent use."

NMS Labs, Willow Grove, Pa., is developing a method including all these different cannabinoids, she says. "Right now, we're telling people if you can do cannabigerol and cannabinol, you have the best chance of identifying recent cannabis use." But other markers need to be studied. "Now the field is developing a lot of cannabis products, including synthetic cannabidiol [CBD], that are high in CBD, and none of these new product formulations have been tested."

**For more than two decades, Dr. Huestis** has been part of a drawn-out rulemaking process to establish federal rules for mandatory drug testing via oral fluid in federally regulated workplaces, such as those under regulation of the Department of Transportation. That effort is near finalization. "I was on the Drug Testing Advisory Board when we started trying to get approval for oral fluid testing in 1994. Now it's very close to being printed in the *Federal Register*." Until that happens, she says, "many people will tell you that oral fluid testing is not federally mandated for their workforce because the final rules haven't come out." But oral fluid is nevertheless being used by millions in nonregulated workplace drug testing.

There are a number of advantages to oral fluid in such settings and in drug treatment, she points out—one advantage being a reduction in specimen collection errors. Saliva offers a much easier way to reduce the adulteration that is so high in urine testing, because oral fluid is an observed sample. In the emergency department as well, oral fluid can be useful. "You don't have to catheterize someone who might have gone through trauma or some other situation, so it's easy to collect and could be a very rapid way of seeing whether drugs may be present, that will help them in their treatment of the patient."

Oral fluid testing targets the parent drug THC, she says. “When you smoke or eat cannabis brownies or other edible cannabis, it gets into your oral mucosa and your saliva from the act of taking it in, so we say it ‘contaminates’ the oral mucosa. THC-COOH in saliva comes from the actual intake of the drug.” THC-COOH, the inactive metabolite that is in the blood, also gets into the oral fluid, but to a much smaller extent—at 1/1,000 the concentration of the parent drug. “The good thing,” Dr. Huestis says, “is that if you do find the metabolite, it indicates the person actually took the drug in; it didn’t just come from a potential environmental exposure.”

Recent papers have shown that people who have a severe environmental exposure—say, from sitting in a cannabis coffee shop in Amsterdam for three hours—could have THC present in their oral fluid without having smoked. But the exposure described in one of the studies was so severe that people wore goggles over their eyes and, after the experience, felt some of the effects of the drug. “There’s probably very little possibility that the individual didn’t know they were in that type of situation, if it’s an enclosed area,” Dr. Huestis points out. “And you’re never going to have a positive result from sitting in a stadium where somebody is smoking nearby; there’s just too much airflow that would be produced.”

Also helpful is the fact that when people are on dronabinol (Marinol), the synthetic THC that’s approved by the Food and Drug Administration, the drug doesn’t contaminate the person’s mouth because it is in capsule form. “So you’d see the metabolite but not the parent,” Dr. Huestis says.

A third useful aspect of THC-COOH is that it enables you to extend the window of detection in chronic, frequent smokers, Dr. Huestis says. “It’s much more present in frequent smokers, because they all have THC buildup in their bodies, and they metabolize it and you find THC-COOH as well.” NIDA has a lot of data on THC-COOH, she adds. “We’ve analyzed it on four different instrument platforms, so if people want to measure it, they certainly have a way to do it. And the main reason would be in cases where someone claims they were not an intentional user but were involuntarily exposed. However, so far the possibility of producing false-positives is low and certainly not with the person not knowing of the exposure.”

A more likely source of preanalytic error is one that tends to protect the individual because it produces false-negatives. For example, use of a stimulant, whether it’s cocaine or cannabis, usually reduces salivary flow. “So that makes it hard to collect a full sample,” Dr. Huestis says. Current federal regulations state that you have to have a full collection or you have to throw the specimen away and start over. “I’m not sure that’s the appropriate way to do it because there are papers showing that short samples have a higher percentage of positive results than full samples, because it means you’re closer to the time of when they used the drug.”

In controlled studies that she has conducted in which people were dosed with drugs, “we tried to get an oral sample, and even after the first half hour with cannabis, you will have a lot of issues. I actually had one person where we couldn’t get a full sample after two hours. The good oral fluid devices all have a volume adequacy indicator. It tells when the minimum amount of oral fluid is collected for that device and if you don’t get it you know you don’t have a full sample.” Unfortunately, efforts to game this system have produced awful urban myths, she says, such as advice to spray one’s mouth with toxic substances like hairspray to reduce saliva volume.

Dr. Jannetto

Specimen collection by lesser trained personnel can be a further error source. “Obviously, for any sample

collection, you need trained individuals. It's much easier to train someone to do good oral fluid collection than to do a good blood collection, but they do need to know what they're doing. They have to follow the chain of custody appropriately, keep the person under surveillance, and make sure they do not have anything to eat or drink 10 minutes prior to collecting the sample."

Mass spectrometry is the closest thing to a gold standard for cannabis testing and helps compensate for many of the weaknesses of immunoassay and oral fluid testing, Mayo's Dr. Jannetto says. "Mass spectrometry gives you that sensitivity and specificity. It also allows you to look at many other analytes and metabolites to differentiate the compounds. So you can look for the parent THC, you can look for 11-hydroxy THC, the other psychoactive component, and THC-COOH. You can also look for other things like CBD, which is not psychoactive but does have more medical use."

In addition, mass spectrometry can look for markers found in natural cannabis but not in synthetics. "But that in itself is a whole analytical nightmare and challenge," Dr. Jannetto says, "because right now there are more than 50 different synthetic cannabinoids on the street, and those formulations change constantly. So unless our assays are constantly updated for new analytes, you could totally miss it. A negative doesn't necessarily mean the patient didn't use a synthetic cannabinoid; they could have used one of the ones I didn't test for."

Just as with any laboratory testing, mass spectrometry's accuracy depends on how the lab sets up and validates its testing, Dr. Jannetto cautions. "Just because somebody says, 'I have a mass spectrometry based-test' doesn't mean it's the gold standard or will give an exact result. The lab still has to have control measures and all of the other things that go along with a properly operated laboratory."

**January 2017—**When the new federal rules are finally published, Dr. Huestis says, they will not allow for on-site screens in the federally regulated workplace. "They require that you have a trained and certified collector collect the sample, which is immediately sent to the laboratory for both screening and confirmation, for the federally regulated workplace in general." However, she adds, there are many employers not federally regulated "that absolutely love roadside or on-site tests. Because you'll be at a construction site, say, and you can screen and if the result is positive, take a second sample and send it to the lab, and avoid hiring the person if it comes back positive."

Drug treatment programs also set a lower standard, Dr. Huestis adds. "They don't in many cases confirm urine tests. But we always say they should confirm if the test will have any negative consequence. For example, in drug court, they'll do the screen, they'll talk to the person, and many times the person will admit use and will have consequences for being positive, although it usually is not going to mean they are kicked out of the program or put back in jail. If they deny using, the treatment programs will run a confirmation test."

The specificity of THC testing is quite good for on-site tests, Dr. Huestis says. "However, obviously anytime there's going to be a potential adverse consequence, whether that's losing your license, losing children, or losing a job, it needs to be confirmed. I think that in most forensic cases, you always have a mass spectrometry confirmation."

The federal rule to regulate cannabis workplace testing came close to publication in 2004, she recalls. "It went all the way through, but it got kicked back when there was a paper that came out and said there could be false-positive results if you weren't smoking but the person next to you was. So it did not get signed; that killed the entire law." But when that particular study was repeated, researchers found that the positive results occurred because they had left all the collection devices inside the area where people were smoking, and the devices became contaminated from the drug in the air. Unfortunately, she says, "That is the kind of preanalytical error source that people seize upon to say there really is a problem."

No federal initiative is underway to make marijuana legal at this point, she notes. "The Drug Enforcement Administration reviews the controlled list every year, and a lot of people, including myself, thought maybe what would happen is they might move THC from Schedule I to Schedule II. Then the FDA would come in and regulate it."

**After doing decades of research on cannabis,** Dr. Huestis believes there are therapeutic applications for the drug, but the big problem is the lack of well-designed, controlled, double-blind studies to demonstrate those uses. “All the things you’d normally have to do to get a drug approved by the FDA—those have not been in place.”

Just in the last couple of months, however, two well-designed studies have shown that Epidiolex, which is a purified cannabis plant extract high in CBD and low in THC, has shown significant results for reducing seizures in individuals who have severe seizure disorders like Dravet syndrome or Lennox-Gastaut syndrome.

Dr. Huestis agrees with the Institute of Medicine recommendation in 1999 that there is now enough evidence that more research is needed to discover potential medical uses. “But we should not be smoking it,” she says firmly. “We need to figure out not only safety but also efficacy, and we need to see how we can standardize potency. Because whenever you have a natural product, it’s difficult to control potency.”

What can laboratories expect from the current climate for cannabis? “I think most hospital and clinical laboratories are going to be in the oral fluid market in the near future, whether from the workplace or from treatment facilities, or in the ER,” Dr. Huestis predicts. “Oral fluid is absolutely coming as a very prominent alternative matrix for the labs. They’re even talking about using it postmortem for autopsy cases. So we will see a lot more test volume.”

“And I think there is going to be a lot more interest in blood cannabinoids that will be of interest to hospital laboratories. It will depend on what the test mix is for the laboratory—many might not do any blood testing for cannabinoids—but blood will be involved where there’s any kind of impairment testing, DUID, or crashes.”

She forecasts that laboratories’ repertoire will be changing. “Right now, labs that are doing blood cannabinoids are doing THC and THC-COOH, and if they’re really good they’re also doing the 11-hydroxy-THC. But I think labs in the future will want to be able to test for some of these other markers of recent use to help them interpret results.”

Also likely to be a big area is therapeutic drug monitoring. “As we get more and more medical marijuana, and you have people on seizure medications, for example, you have to know whether they’ve got adequate concentrations to prevent the seizures,” Dr. Huestis explains. Studies of marijuana as treatment for neuropathic pain and migraines are underway. “So they will start doing therapeutic drug monitoring to test what the concentrations of THC are and whether they’re in therapeutic ranges.”

Amid the expanding universe of cannabis testing, Dr. Jannetto warns, people must continue to be aware of one of the core features of any laboratory test: limitations. “The testing that is locally available for law enforcement or physicians—they are going to use whatever devices are accessible to them. They have to understand the limitations of the technology and test.”

For laboratories, “given the new legal frameworks, the key issue is understanding the limitations of different matrices, what information you can draw from each specimen type, what analytes you are looking for, and which biomarkers are actually correlated with impairment or use and which aren’t.” Keeping those limitations in mind, Dr. Jannetto says, will help ensure that all types of cannabis testing are properly interpreted and used.
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