

Host: This is the podcast from *Clinical Chemistry*. I am Bob Barrett. Using oral fluid saliva to monitor licit and illicit drug use is increasing rapidly throughout Europe, North America, and Australia. There currently is great interest in this alternative matrix for documenting driving under the influence of drugs, and for workplace and drug treatment testing.

Although, therapeutic drug monitoring of oral fluid offers a noninvasive means of estimating free drug concentrations in plasma or serum, this technique is infrequently used. An article in the November issue of *Clinical Chemistry* by Dr. Nora Badawi and co-authors addresses drug testing in oral fluid, and an editorial by Dr. Marilyn Huestis in the following issue further explores this method, which could aid clinical chemists and toxicologists who are interested in implementing oral fluid analysis.

Dr. Marilyn Huestis is the Chief of Chemistry and Drug Metabolism Intramural Research Program, National Institute of Drug Abuse at the National Institutes of Health, and she is our guest in this podcast.

Tell us Dr. Huestis, why is drug testing of oral fluid samples an important topic right now?

Dr. Marilyn Huestis: Well, oral fluid testing has become the hot new topic for biological monitoring of drug use in multiple areas, probably the area that's led the field is the area of driving under the influence of drugs or human performance testing. And the Europeans are quite far ahead of those of us in the Americas with this effort, with the European Union putting a lot of funds behind trying to come up with a way to monitor drug driving similar to what we do with alcohol-impaired driving.

The ROSITA Project was the first effort in this regard and they looked at urine, oral fluid, blood, and sweat and based on that very expensive study that was run in multiple European countries, they determined that oral fluid was the most promising matrix for monitoring at the roadside and for impairment. But at the time the technology was not in place to do it well, that the technology needed further development.

In the second ROSITA project, the United States took part as well, and again, oral fluid samples at this point where they were comparing multiple new products on the market from many vendors around the world. And again, they didn't feel that the oral fluid testing had evolved to the point that they could start using it routinely and now the Europeans have the DRUID project, which has selected a particular device and is going on with roadside monitoring in many

European countries with the goal to reduce drug driving deaths by 25% in the next few years.

Now that's one aspect. Another is for workplace drug testing. And so in the United States the Substance Abuse Mental Health Services Administration has been working for the last 15 years allowing additional matrices other than urine for drug testing and the matrices under consideration are oral fluid, sweat, and hair, and oral fluid testing has made tremendous leaps and bounds in this area as well.

So the subject of the editorial that we are discussing today is a new method under the DRUID program to monitor a large number of specimens. So we've made both technical advances as well as advances in understanding the factors that relate to the disposition of drugs into oral fluid.

Host: So what are the advantages and disadvantages of oral fluid for the monitoring of drug exposure?

Dr. Marilyn Huestis: Well, there are many on both sides. The most interesting one is that you can take an oral fluid specimen, for instance, right at the roadside it's completely observed. There is no loss of privacy involved with taking the oral fluid sample. We believe that the adulteration techniques that are so prevalent with urine drug testing and that are causing tremendous problems in that regard, even the simple drinking of large amounts of fluid can reduce drug concentrations in urine below the cut-off concentrations.

In oral fluid you are actually observing the individual give the specimen, it's more difficult to adulterate, and it's something that can be done by either men or women taking the sample from an individual, where with urine drug testing you have to have a gender-specific individual accept the specimen. And the other issue that's very interesting is that it has less danger as far as transmittable materials than blood. So that's another advantage to oral fluid.

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And then, interestingly, depending upon the purpose or goal of your testing, the fact that it has a shorter window of drug detection than urine can be positive.

So for instance, if you are looking at roadside drug testing and drug-impaired driving, an individual can be positive in the urine for a very long period of time beyond the intoxication window. And the oral fluid window of detection is closer to that of blood, it's very similar the windows of detection to blood and potential intoxication.

But if you are looking at workplace drug testing where you might be interested in looking at the longest window of detection it might be a disadvantage to oral fluid testing as compared to the intoxication. And also for the area of drug treatment oral fluid testing has some real advantages because in drug treatment, if an individual comes in on Monday and is positive for cocaine in the urine, they will take different steps in counsel with the individual. They may involve dosage differences in the methadone or buprenorphine treatment the individual might be receiving, for instance.

And then the person comes back on Wednesday and they are still positive. We don't know since they are doing qualitative testing whether or not the individual relapsed again between Monday and Wednesday because of the long detection window in urine. Whereas if you take oral fluid the window is shorter and more likely that if you find a positive on Wednesday they relapsed again. So it can help with interpretation of testing as well.

Now there are some advantages, if you are looking at basic drugs. So for instance, like cocaine or methamphetamine that are big problems in our country, they tend to become increased in higher concentrations in oral fluid as compared to blood, and that process is called "ion trapping."

When you have a basic compound in blood to get it transferred across the plasma membrane into oral fluid the more lipophilic the drug compound is the easier it will cross, and if it is not charged, if it's in the neutral state, it will cross over as well.

But what's interesting is that the pH of oral fluid is usually less than the pH of blood. So you have this process of ion trapping where the drug, a base that's in the neutral state in blood will cross over and form an equilibrium across the barrier, and then when it gets into the more lower pH oral fluid it will ionize and become what we call trapped in the oral fluid and then this will drive the concentration across that plasma membrane.

So you end up with higher concentrations in oral fluid than in blood, and that makes it easier to detect. And because in general, you have the more lipophilic, more neutral compounds in oral fluid, that's also usually the parent drug.

So in oral fluid you actually frequently are measuring parent drugs as compared to in urine you are measuring usually metabolites, more polar metabolites. So that again helps with the issue of your actually measuring the active drug component rather than a metabolite, which helps in your interpretations of the concentrations.

Now as far as disadvantages, it also has some. Every matrix has disadvantages. One of the issues is the fact that you get a lot less specimen when you collect oral fluid than when you collect urine. You have a lot larger specimen volume with urine. So this is an issue about being able to test for a large number of drugs when your volume is small.

Another disadvantage is that the actual secretion of oral fluid changes dramatically based on whether the oral fluid secretion is stimulated. And when you take stimulants like cocaine or methamphetamine or amphetamines they can reduce stimulation of oral fluid and reduce the amount of drug that's actually secreted. So that can be a problem.

If you use a collection device that has citric acid or some other chemical that causes increased secretion, what we find is twofold, we find a large volume of oral fluid that's secreted, which certainly helps as far as the amount that you are collecting. But in reality it's diluting out the concentrations of the drugs, and this can be problematic as far as your detection limits.

Another thing that we've learned since oral fluid testing has been under all of this scrutiny more recently is that when you stimulate oral fluid, you also change the pH.

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When you stimulate oral fluid the amount of bicarbonate that you excrete increases, and when it increases, now the difference in pH between blood and oral fluid is less because you've raised the pH in the oral fluid. Therefore, you have less ion trapping and lower concentration. So that's certainly a disadvantage.

And then we'll talk a little bit later about the oral fluid collection devices and getting those optimized has been a real issue and problems and certainly will be listed under the disadvantage of oral fluid until just recently with improvements.

Host: So what factors influence the secretion of oral fluid?

Dr. Marilyn Huestis: Well, first of all other drugs that you might be taking at the same time can either increase or decrease the secretion of oral fluid, and we gave the example of the stimulant drugs do produce dry mouth and reduce that. Also marijuana or cannabis can reduce the secretion. And also whether you are chewing or taking food that can increase it, or even seeing something like seeing good food could increase the secretion.

Host: Why is LC-MS/MS, or liquid chromatography tandem-mass spectrometry, particularly suited for oral fluid analysis?

Dr. Marilyn Huestis: Well, initially LC-MS can look at a broader spectrum of drugs than GC-MS. So drugs that are not volatile, however, that are thermal labile can be decreased with GC-MS, whereas with LC-MS you don't have that problem. And LC-MS also permits you to look at it. We have a wide variety of different techniques that we can use with LC-MS that can look at a broader spectrum of drugs.

And what's very interesting is LC tandem-mass spectrometry is particularly suited because you can actually screen for a large number of drugs. And in fact, many different locations and laboratories are looking to eliminate screening methodology and actually do their screening not by enzyme-immunoassay or by GC-MS but trying to do screening with LC-MS/MS, and then for confirmatory procedures maybe have more specific selective LC-MS/MS procedures.

Host: What are the strings of this new UPLC/MS/MS oral fluid assay?

Dr. Marilyn Huestis: One of the main problems or issues still to be resolved with oral fluid testing is the collection device themselves. There is a wide variety of oral fluid collection devices on the market. One of the biggest issues is the amount of oral fluid that they actually are able to collect. Some of the devices now with great improvements over the last five years are measuring a reproducible amount of oral fluid.

In the past, the amount collected varied tremendously, that was one major problem. And second of all, they didn't collect enough. So now devices are on the market that can collect 1 ml within about 10% variability. And the third issue was that these devices, many of the drugs, especially the more lipophilic drugs actually bound to the device itself and it was very, very hard to extract it off of those devices.

This particular new oral fluid assay has a number of strengths. One being that they actually weighed the devices prior to collection of the oral fluid, and then weighed the device after collection. So they have a good idea of the amount of oral fluid that was collected. This helps and that they can then normalize the results based on the amount they collected to give you a better idea of actually the drug concentration.

In general, the cut-offs that have been put out by different regulatory agencies like the SAMHSA organization or the DRUID organization have based it on volume measurements rather than weight. But in general, the amount of drug is

quite equivalent to the volume of drugs for oral fluid. It has different densities, but in general they are pretty much like water so you can make that conversion. So that was one strength of this method.

The fact that they were able to screen and quantify 29 different important drugs that are involved with drug-impaired driving was another major strength.

One of the disadvantages of LC-MS/MS compared to GC-MS is the factor of matrix effects, and matrix effects can be very important and in your method validation procedures, it's key that you perform very good analysis of matrix effects.

Now by using deuterated internal standards you can compensate for most of these effects.

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And so if you do find ion suppression or ion enhancement, the fact that you have a deuterated internal standard present is extremely helpful for compensating for those effects. It doesn't entirely remove them but it compensates for them.

So of the 29 drugs that were included in this assay, they had 25 deuterated internal standards. Now that's a little expensive to do, but by screening for so many different drugs within one procedure, they were able to reduce the cost of analysis of multiple procedures by being able to do it all in one.

So that was another major advantage to this particular method. They had good method validation procedures. There were a few that maybe could have been a little stronger but they had quite a very good method validation procedures in place. And also the fact that they were able to quantify and had good linear ranges available that would prevent the need for multiple dilutions in such a large wide scope broad screening program, was also another strong advantage.

Host: In your opinion, are there issues with using synthetic oral fluid for the preparation of oral fluid calibrators and controls?

Dr. Marilyn Huestis: Well, this is one of those important research questions that we don't have answers for at the current time, but another strength of this manuscript was that they actually compared the concentrations that were fortified into authentic oral fluid and into synthetic oral fluid. And with the particular synthetic oral fluid that they were using they found very

large differences in matrix effects between the two. Now this is really important.

If you have a synthetic oral fluid that you were using for your calibrators and for your controls, because your quantifications will be quite different due to matrix effects when you actually measure authentic human specimens.

So this is another strong warning sign to anyone going into oral fluid testing and using LC-MS/MS, how important your method validation is, and the fact that if you are going to try to use synthetic oral fluid for the basis of your standards and controls that you must fully validate and look for issues of matrix effects that could certainly have a strong effect on your quantifications.

Host: Can blood concentrations be predicted from oral fluid concentrations?

Dr. Marilyn Huestis: Well, though we have another major issue. So as we improve the technology of both the oral fluid collection devices and we have improved our analytical methods for actually quantifying and we've learned about the issues of oral fluid testing, we still have the problem as how do you interpret these results.

And this is one of the areas, since it is a new very important area in clinical chemistry, in clinical toxicology, we need the controlled drug administration studies to be able to guide our interpretation. And we need more research in this particular area.

In my laboratory we have been doing a lot of controlled administration of both licit and illicit drugs to try to come up with a scientific database to guide and help in this interpretation issue.

So one of the major issues is that if countries or states include legislation permitting oral fluid testing and many states and countries have done so, including in the United States, allowing oral fluid testing, do oral fluid drug concentrations accurately predict blood concentrations in the same individual?

And from our work with controlled drug administrations, we have seen that although oral fluid concentrations and blood concentrations temporarily correlate very well that there is enough both intra and inter-subject variability that you would never want to try to predict a specific blood concentration from an oral fluid quantification.

Now, this is an area that's very controversial right now and that many people would like to be able to do this so that the

laws in their country that are directed toward blood concentrations could be seen as equivalent to oral fluid concentrations. And this is going to be difficult to do, and I think that in the end that although again, they are temporarily correlated, you should base your interpretation on the oral fluid level itself and not try to predict what the simultaneous blood concentration would be. But this is an area of active interest at this point.

Host: Which are the most difficult analytes to measure quantitatively in oral fluid?

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Dr. Marilyn Huestis: Well, there is a number of different classes that are difficult. One are the benzodiazepines. So the benzodiazepines, which is an important class of drug, in fact, there are some of the most prescribed drugs, the highest number of prescriptions in the world, they get into oral fluid and low concentrations, especially the highly potent drug compounds that are already present in blood in very low concentrations very difficult to get those into oral fluid.

Also drugs metabolites that are more polar as we spoke before, if they are either polar, they are large molecule or they are highly charged, they are not going to get across the membrane. So there is a wide variety of different types.

So I'll give you an example of Cannabinoids or cannabis or marijuana. So the parent compound THC, Δ^9 -Tetrahydrocannabinol gets into oral fluid very readily from the contamination that occurs during smoking, because of course, when you smoke a drug and many different drugs are smoked, they get into the oral fluid in very high concentration, and then they clear from the oral fluid, from these very, very concentrations within about 45 minutes or an hour, and then you see the good correlation with blood concentrations.

But for instance, the metabolite of THC, the 11 or 9-carboxy-THC, that is present in oral fluid only from drug that comes from the blood and then passes into the oral fluid. So it is present in very low concentrations in picogram per ml concentrations rather than nanogram per ml concentrations.

Well, you might ask, why is that important? Well, it's very important because the THC you don't know, you have the potential for it coming from passive exposure in the environment.

So for instance, if you're not smoking cannabis but your neighbors are or someone in a car is, it's certainly possible

that that drug can get into the oral fluid, and we don't know yet, whether or not it would produce a false-positive result.

We have some indications that say it's possible, another indications say it's not highly likely. But if you could measure the metabolite in this case, you would be sure that the individual actually used the drug. And in our controlled THC administration studies that we are currently carrying out here at NIDA, we have been able to show that if you can get a good method with low detection limits, which we have in picogram per ml levels, you can clearly eliminate the potential for passive contamination.

So that's going to be very important, I think, in a number of smoke drugs to be able to measure metabolites that are not present in the actual drug smoke.

Host: Well finally, what areas of oral fluid testing would benefit most from some additional research?

Dr. Marilyn Huestis: I would say all aspects are really in need of additional research. Certainly we've made big headways in the oral fluid collectors and we're making good headway on analytical procedures, but certainly with this issue of a synthetic oral fluid perhaps having a strong matrix effects differences, that needs to be looked into.

We need to validate all of the collection devices and determine if they can collect sufficient volume of oral fluid in a reproducible manner. We need to make sure that our oral fluid collection devices that we don't have drugs absorbing strongly onto them, that we are using buffers that will release these drugs so that we can measure them effectively.

We need additional procedures from many drugs. We need lots of controlled drug administration studies to be able to point to not only what analytes we should be measuring, what kind of concentrations that we should be expecting and how they correlate with drug intoxication and impairment.

So the whole area of oral fluid testing is certainly a need in benefiting tremendously from additional research.

Host: Dr. Marilyn Huestis is the Chief of Chemistry and Drug Metabolism Intramural Research Program, National Institute of Drug Abuse at the National Institutes of Health. She has been our guest in this podcast from *Clinical Chemistry*.

I am Bob Barrett. Thanks for listening.

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