PROCEEDINGS

Substance Abuse and Mental Health Services Administration (SAMHSA)
Center for Substance Abuse Prevention (CSAP)
Drug Testing Advisory Board (DTAB) MEETING

January 27, 2011

One Choke Cherry Road
Rockville, Maryland 20857

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P R O C E E D I N G (8:30 a.m.)

Call to order

Dr. Cook: Good morning. I am Janine Denis Cook, the Designated Federal Official of the Drug Testing Advisory Board. At this time, I am calling the morning session to order.

I have a few announcements. Anyone on-site who has not yet signed in at the back registration table, please do so now. Also, those of you that have flight arrangements today, we ask that you please check to make sure that your flights are on time. We will try to help you any way we can, if you need to make other arrangements. For those of you on-site, please wear your badge when you are in the building. At the end of the day, please return the badge to the guard station. For those of you that are new to us today on-site, the bathrooms are located outside the door to your right. We also have a break room that houses the Uncommon Café, which is also located o outside the door to the right and down the hall. At the back table is the agenda for today.

For those of you who are off-site, I have some information for you about the Adobe Connect. I want to thank Erica and Jared, our Adobe Connect hosts. The meeting logistics has been very difficult because of the weather. Many people who originally planned to attend outside are now on-line. Jared and Erica did a Herculean effort -- I do not think Jared slept last night -- to make sure that everyone could connect in. Erica has provided me with instructions regarding Adobe Connect. First, I will point out a few things about the room that you are viewing. Most of the presentations will be shown through our share pods today. These will be the largest pods and will take up most of your screen. The Attendees List pod displays the list of everyone in the room with us today. At the top of the Attendees List, there is a My Status dropdown arrow. These My Status options are a great way to communicate with us. For example, the Stepped Away status lets us know that an attendee has temporarily stepped away from the room and the Raise My Hand status lets us know that you have a question. The Chat Pod allows you to communicate with us at any time concerning either a technical problem or pertaining to the material. There is a white bar at the bottom of the Chat Pod. You simply take your cursor and click in that white bar. This will allow you to begin typing your question and then you click enter to send your message. All questions submitted pertaining to the material given will be taken under consideration by the Board in the closed session. If you have any technical problems, please submit them in the Chat Pod. This is a virtual meeting room with a maximum capacity of 100 attendees. Please note that only those participants who are logged into the room will be able to submit questions. Participants who are just calling in will not be able to provide comments.

For anyone on-site who has a questions regarding material that is presented, you can write it down and submit it to someone at the registration table. It will be taken under advisement by the Drug Testing Board during the closed session. We will not answer any questions at this time.

We do have one public comment from one public commenter who had registered to give comment yesterday but because of technical difficulties, he was unable to log. He has sent it to me, and at this time, I will to read it.

My name is Steven Soifer, and I am the CEO of the International Paruresis Association (IPA) and an Associate Professor of Social Work at the University of Maryland. The non-profit organization, which I co-founded, has over 1,500 members worldwide and helps people who suffer from the social anxiety disorder and chronic pelvic floor dysfunction better known as shy bladder syndrome. Currently, there are an estimated 17 million people in the United States who suffer from this social phobia or about seven percent of the population. Most who suffer cannot urinate on demand for a drug test. You may

ask “where are all these people?” We do not see many of them. That is maybe true, but I guarantee you, whether through self-selection or getting by somehow, they are there by the thousands. This is another form of Don’t Ask, Don’t Tell. In July 2004, the International Paruresis Association sent a formal letter of comment regarding SAMHSA’s then-proposed new regulations regarding drug testing of Federal employees and implementing alternative drug tests, such as hair, saliva, and sweat testing. IPA members, 1,500 strong, also sent in hundreds of public comments received by SAMHSA/HHS on the new proposed regulations. In our previous formal testimony, the IPA reiterated our position that we are not opposed to drug testing, but that we are merely asking that the new regulations be worded in a way that allows alternative testing for those who are unable to provide a voluntary urine specimen due to Shy Bladder Syndrome. SAMHSA drug testing rules currently rely exclusively on urine testing and do not permit alternative sample testing for those who suffer from paruresis or other medical conditions, which may cause an unexplained inability to produce a urine sample. We have been unable to get any assistance from anyone in SAMHSA or at DTAB. There is a real understanding of the problem in the new rules that may cause job seekers who are unable to produce a urine sample, including those who apply for non-government positions and positions regulated by DOT. The present reliance on urine-based testing to the exclusion of other variable options is unacceptable to us. As it is the insistence that a failure to produce a specimen equates with refusal to be tested. IPA has several requests regarding these regulations. We want DTAB/SAMHSA to officiate knowledge that the problem of paruresis is real and assure that reasonable accommodation will be built into the testing rules. We want DTAB/SAMHSA to mandate the use of hair, saliva, or oral fluid in lieu of urine for anyone unable to produce a urine sample within the two hour time limit. We believe that SAMHSA and HHS drug testing standards are used as the gold standard and influence drug testing procedures currently used by DOT and many commercial companies. We therefore ask for SAMHSA’s understanding in working with us on the drug testing problems for the paruresis community. We hope that meetings will be set up on an on-going basis with SAMHSA and our organization. We are and always have been willing to work with SAMHSA to help it address our legitimate concerns and the need for scientific, valid testing of employees. On September 26, 2008, Congress passed Law S.3406 or the ADA Amendments Act. Most importantly, the amendments, which took effect January 1, 2009, clarify that disabilities include major bodily functions, including those of the bladder. It is now illegal to discriminate against anyone with bladder problems, which clearly includes shy bladder. Consequently, reasonable accommodation must now be provided for people with paruresis, including in all Federal agencies. We ask that DTAB immediately take this under advisement and take the necessary steps to provide reasonable accommodations in the drug testing arena by making alternative testing like saliva and hair available to shy bladder sufferers. The EEOC will be issuing their rule interpretations for the ADAAA very soon so now is an appropriate time for DTAB/SAMHSA to act. Thank you.

Oral Fluid Matrix - Historical Perspective

Dr. Cook: We will now proceed with the rest of the agenda. I would like to call Dr. Michael Walsh, who will be the historical perspective of oral fluid testing.

Dr. Walsh: Thank you, Janine. Welcome, everybody, to sunny Gaithersburg. My job this morning is to, in a broad brush way, set the scene for the in-depth talks that follow. I will begin with a brief history of the HHS Guidelines development, which you heard about yesterday, then alternative matrices, and finally, the current state-of-the-art in oral fluid testing, including the comparability of oral fluid to urine in detecting drug use. I will begin the discussion today about how oral fluid might fit into the Federal Guidelines paradigm.

In September of 1986, President Reagan signed Executive Order 12564. The Secretary of Health and

Human Services was tasked to develop the Guidelines. The Secretary appropriately delegated this to Bob Schuster, the Director of NIDA, who promptly delegated the task to me.

We issued an initial version of the Guidelines in February of 1987, which contained the standards for collection, laboratory testing, etc., but it did not include laboratory certification. At a meeting held at the HHS headquarters downtown, Congress got nervous that the government would actually begin to drug testing people. Between February and July, there were a series of meetings, which precursors to this Bill that the Congress passed, Public Law 100-71, which developed the prerequisites that must be completed before any appropriated funds could be used to drug test any Federal employee. One of those prerequisites was to revise the Guidelines to include a laboratory certification program, and to publish in the Federal Register the elements of this program for public comment. We were also required to assist every Federal agency, all 186 of them, in developing its agency drug testing plan that included what employees were going to be tested. It took us a little while to complete all of the prerequisites. The final notice of the new Guidelines for Federal employees was issued in April of 1988, and it now included standards for collection, screening and confirmation testing, and a certification program for laboratories.

When the Guidelines were originally issued, it was focused on Federal employees. Actions by the Department of Transportation, the Nuclear Regulatory Commission, and other federal agencies to adopt those for use in their own regulation of the industries that they regulate significantly expanded the impact of the Guidelines.

Over the next 15 years, from 1988-2004, the Guidelines were fine-tuned. When the Alcohol, Drug Abuse, and Mental Health Administration was abolished, NIDA, NIAAA, and NIMH were moved to NIH and this program relocated to SAMHSA, which had been newly created.

Subsequently, in 2004, SAMHSA proposed changes to the Guidelines to include alternative matrices -- oral fluid, hair, and sweat. Four years later, there was a Final Notice in 2008 which stated that urine would remain the only approved specimen matrix for Federal programs because of the public comment and Federal agency concerns. This comments indicated that the technology for hair, oral fluid, and sweat was not sufficiently mature to include in the Federal program at that time. Over the last three years or so, more research has been done oral fluid and the science and technology for detecting drugs in oral fluid seems to have reached the point where further consideration appears to be merited.

My goal is to begin a process of discussion to identify the issues concerning the possible inclusion of oral fluid in the Federal testing program. What you will hear for the rest of the day are some of the technical, regulatory, lab certifications, and MRO issues, and the legal defensibility of oral fluid; these are considerations for any of the alternative matrices. Though we evaluated oral fluid in the early part of this last decade through the various working groups and DTAB, the increased experience has really highlighted what some of the really nitty-gritty issues are.

When choosing a specimen matrix for workplace testing, there are many variables to consider. What is the reason for the test? How often are you going to test? What is the window of detection that is required? What drugs are you going to test for? Do you need immediate results? s there a device and an assay available to do what you want to do? What is the venue? Where are you going to be testing - on the desert in Afghanistan or in the staff clinic here at SAMHSA? There are so many questions to consider.

I have been working in the drugged driving field which has uncovered many logistical issues, such as

performing a point of collection test on the side of the road in Norway in the dead of winter to reading results in the pouring rain. These are some of the issues that you have to consider.

This slide, borrowed from Yale Caplan and Ed Cone, depicts the window of detection for the various matrices. The timelines for blood and oral fluid are somewhat similar and much closer to tying the results of a drug test to impairment. Urine then sweat and finally hair provide much longer windows of detection.

There are pros and cons to using oral fluid, like there are with all of the matrices. It is clearly less invasive than urine, provides evidence of very recent exposure, and contains the active drug rather than the metabolite. There are laboratory assays and point-of-collection test devices that are available to test for oral fluid. The cons include the window of detection is shorter than urine, although the lab window of detection is better than the point-of-collection test; the collection method is critical; and the contamination issues. This oral fluid collection concern will be addressed in the discussions later in the day about how to collect an oral fluid.

Regarding assay availability, there are many new assays that are awaiting FDA approval. The point-of-collection tests that are available are only really accurate for some drugs. This I will discuss more and share some data with you.

Oral fluid in drug testing has been around for quite a while. Studies reporting the detection of drugs in oral fluid date to the early 1970's.

RTI has developed a 29 page bibliography of oral fluid studies. The oral fluid specimen is widely used in therapeutic drug monitoring, pharmacokinetic studies, and in the detection of illegal drug use. For quite a number of years now, laboratories have specialized in testing for risk management in the workplace/business and insurance companies, etc. Oral fluid is used to test for cotinine to determine if you are a smoker, for HIV, and illegal drug use. There is a fair amount of data now available.

For lab-based testing, assays are available. The ELISA method has been available for years. The heterogeneous assays for most drugs are available. As you heard in Dave Kuntz' public comment yesterday, the new oral fluid enzyme immunoassays are homogenous and fully-automated and are becoming available. FDA is expected to approve most of the drugs of interest for this kind of work.

For confirmation assays, the GC/MS has been the workhorse for many years, but many of the laboratories are now using LC/MS/MS, especially for therapeutic monitoring for pain management using oral fluid. Thus, data exist for the opiates, both natural and synthetic, in oral fluid.

For point-of-collection tests, there are a number of devices available. Most are visually interpreted, although some utilize available readers. The analytical specificity and sensitivity and accuracy depend upon which drug you are testing for and on what device. In general, the data are pretty good for amphetamine and methamphetamine but only okay for opiates. The other drugs of interest have less than desirable analytical performance, which I will show you. My personal conclusion on the oral fluid point-of-collection tests is that the overall performance is poor when you compare it with laboratory-based oral fluid testing.

There data on point-of-collection tests are derived from the ROSITA (Road-side Testing Assessment) II project, a joint U.S. and European Union field evaluation of point-of-collection oral fluid test devices that collected data from nearly 2,300 DUI suspects. These were individuals were stopped by police in four U.S. states and six European countries that were involved in project. For the study, blood and

two oral fluid specimens were collected from each of the DUI suspects. An on-site point-of-collection test (POCT) was employed for one of the oral fluid specimen test results, This POCT result was compare to the result of oral fluid that was analyzed in a laboratory If the results concurred, no further testing was performed. If not, the blood test was performed to serve as the gold standard reference.

In this slide is the overall positivity rate from this population of DUI suspects from the lab based on lab tested oral fluid. A little more than a third of them were positive for THC. The benzodiazepines were much higher in Europe than they were here in the United States, but amphetamines, cocaine, and opiates all found in pretty substantial numbers.

We evaluated ten different on-site point-of-collection tests, some of which are no longer available. The Drager Uplink has been replaced by a newer and better model. I don't believe the Lifepoint is still in existence. Whenever we published the poor performance results of one of these devices, the manufacturer soon release a new device that was touted to be much better and more sensitive. Because these devices are not as good as lab-based testing does not mean that there aren't people out there using them. The Australians are using the Securetec Drugwipe for their random DUI stops of drivers all over Australia. The Belgians, who recently switched from urine to oral fluid, are using the Securetec Drugwipe as well.

There were only two devices that did really well, meaning they performed well all the time regardless of condition, the degree of training of the operators, etc. Those devices highlighted in yellow had a less than 10 percent failure rate. The rest the devices had high failure rates because the tests would stop and not run to completion, especially the lateral flow devices. Our conclusions are that the laboratory analysis of oral fluid was excellent. The point-of-collection oral fluid drug tests were less accurate. As seen in this last slide, six out of ten devices had failure rates greater than 25 percent. For marijuana testing, the point-of-collection tests only detected 50 percent of the positives that were identified by lab-based testing. The detections rates for the other drugs were 80 percent for amphetamines and cocaine, 67 percent for the benzodiazepines, and 60 percent for the opiates. There were many false negatives with the point-of-collection tests.

My group has been working with RTI and the National Laboratory Certification Program for the last seven or eight years. We have analyzed the unregulated drug data from two million urine and 650,000 oral fluid tests from a single large MRO source to compare oral fluid with urine. All test results were included in the database except for all blind QCs samples. The oral fluid specimens were collected during a five-year period between 2003 and 2007. During this time, there was a dramatic growth rate in the numbers of workplace oral fluid specimens reviewed through this particular MRO. The majority of the oral fluid specimens were analyzed by two large laboratory chains. The overall positivity rate for the tested 650,000 oral fluid specimens was a 4.3 percent with MRO-verified positivity rate was 95.6 percent. Thus, only 4.4 percent were reversed in the MRO process.

This slide breaks down the distribution of the MRO-verified positives by drug. The positivity rate for marijuana is 60 percent, which is somewhat surprisingly because of the relatively short window of detection for marijuana. The rates for the other drugs were 24 percent cocaine and 6.4 percent for methamphetamine, followed by amphetamine, opiates, and not surprisingly, PCPs.

The two million unregulated urine tests, collected between 2006 and 2007, yielded a sufficient number to compare with the five years worth of data on oral fluids. These specimens were all analyzed in the SAMHSA-certified laboratories. This slide gives a side-by-side comparison of the two specimen types, which are not matched samples because they are different people.
Generally, the frequency distributions are pretty close. The oral fluid assay appears to detect a few

more cocaines and methamphetamines than urine. The overall positive rates were 4.15 percent in lab-confirmed results in urine and 4.3 percent in oral fluid. The one big difference was that the MROs were reversing more urine positives than oral fluid. We discussed this and could not ascertain why that was true. Overall, the lab-positive rates are comparable between urine and oral fluid. The MRO-positive rate was higher for oral fluid. For both specimens, the majority of the MRO reversals appear to be due to prescription use of opiates and amphetamines. The reversal rate in urine during the seven years of this project has increased. In 2003, the reversal rate in urine for amphetamine positives was about 20 percent; it has grown every year and this last year it with 85 percent. I suspect that Adderall is one reason for this increase. The pharmaceutical industry has, over the same time period, dramatically increased their marketing of Adderall for adults.

To summarize, the Guidelines have survived nearly 25 years. The technology has changed significantly. This provides a major opportunity now to improve the Program and increase efficiency and cost-effectiveness. The early Guidelines were based on technology that was available in the middle 1980's, which was a long time ago. Technology has changed dramatically. To add alternative matrices to the program, we need to think outside the box and not try to fit another specimen into the urine paradigm, all the while maintaining the quality control, legal defensibility, and confidence in the program. Today is the beginning of the process to inform and discuss the current state-of-the-art of oral fluid drug test methods to explore the suitability for the Federal program. Thank you for listening.

Dr. Cook: Do any of the Board members have questions for Mike? Thank you, Mike, for providing a historical perspective of oral fluid.

Oral Fluid Matrix - Current Perspective

Dr. Cook: Now we change our focus to examine the current state-of-the-art, as we know it, for drug testing in oral fluid. I would like to introduce Dennis Crouch, who will discuss the oral fluid matrix specimen. Denny is not here on-site, so he will join us from Utah.

Specimen

Mr. Crouch: Thank you. Good morning. I will present some data on the background of how saliva is formed and how drugs are transferred into saliva. Next, I will discuss the kinetics of drugs in oral fluid versus plasma, the effects of collection techniques on oral fluid concentrations, and the validity testing in oral fluids. I am presenting these concepts as teasers to stimulate our thinking about what we need to consider in formulating guidelines for oral fluid testing.

The use of oral fluids for drug testing goes back years. It was used in anti-doping efforts for the detection of opiates as early as the 1900's in Europe. It is also used in clinical applications where most of the history regarding the use of saliva or oral fluids resides. This specimen is advocated for DUI and DUID, the workplace, and for drug treatment, probation, and parole.

One of the unique and potentially dynamic features of oral fluid is that the oral fluid drug concentration should or can reflect blood drug concentrations because oral fluid is really an ultrafiltrate of the blood. This has advantages in therapeutic monitoring and is currently being used in pain management. There are also some significant advantages in the collection of oral fluid versus blood or urine, including that the collection can be observed, it is non-invasive, multiple samples can be collected one right after another, and the collection does not involve a venipuncture as with blood or some of the issues that are involved in the collection of urine.

The nomenclature for this specimen has alternately defined as saliva, oral fluid, oral fluids, whole saliva, mixed saliva, etc. These terms are used interchangeably in the literature and are defined in different ways in the literature. For example, some leading resources have defined saliva as an expectorant and others say that saliva is collected from a specific gland.

Shown in this slide are the three major glands that produce saliva: the submandibular, shown with the yellow arrow below the mandible; the sublingual, below the tongue; and the parotid. There are also some minor glands that are involved in saliva production, such as the labial, which is indicated here in green. Remember, though, the literature varies about the source of saliva. In mixed saliva, which is referred to as oral fluid, roughly 65 percent is from the submandibular, 23 percent from the parotid, 4 percent from the sublingual, and the rest from these minor saliva glands.

The next topic is stimulated versus non-stimulated saliva or oral fluid. Stimulation occurs usually by one of two mechanisms, mechanical, which means chewing, or chemical, such as a citric acid from a lemon drop will stimulate oral fluid production.

Oral fluid production can reach up to 1.5 liters a day. The pH of oral fluid is roughly one pH unit less than blood and can vary by a pH unit from six to eight. Saliva is primarily 98-99 percent water. Our saliva production when asleep is about 50 microliters a minute. Because spitting stimulates oral fluid production, saliva production when spitting is about 0.5 mL a minute. Chewing, such as gum, paraffin, or rubber bands, stimulates oral fluid production at a rate of about one to three mL a minute. If we stimulate chemically with citric acid, production can increase to five to ten mL a minute.

The common electrolytes found in plasma are also found in oral fluid but in different concentrations. For example, potassium concentrations are roughly two to eight times those of plasma, while sodium and chloride are 5 to 70 percent those of plasma. There is a reason that occurs and that is shown in the next slide.

This is how saliva is formed. Shown in the bottom left of this slide is the entry of arterial blood (red arrow) into and the bed of capillaries around the duct of the gland. The blood flow occurs across the head of the gland into the bed of capillaries and then flows out as venous blood. The primary functional area of the gland is the head, which contains acinar cells. Acinar cells are very permeable to water so this is where most of the transfer of drugs occurs. Potassium, chloride, sodium, water, and bicarbonate enter the salivary gland at the head. The saliva then passes down to the ductal portion of the gland and into the buccal cavity. Note the arrows, especially the bold arrow for sodium. Sodium is actively re-absorbed into the blood and poorly absorbed back into the duct. For potassium, concentrations can be higher in the duct as noted by the arrow. The reabsorption differences explain the differences in concentrations in the previous slide. The other thing that is shown here is bicarbonate. As we increase the saliva flow, our ability to reabsorb these electrolytes, if you will, decreases so things like bicarbonate increase and that changes the pH of the oral fluid, which is particularly important in terms of the distribution of the drugs into the oral fluid.

What analytes do we find in oral fluid? We find the electrolytes - sodium, potassium, chloride, and bicarbonate – and lithium; calcium; immunoglobulins; steroids; various enzymes; especially amylase; DNA; viruses; etc. Immunoglobulins are important because IgG and IgA have been suggested as markers of specimen validity. What can also be present in oral fluid are drugs, especially those of importance to us, such as the drugs of abuse, those for DUI, or for other purposes, including alcohol, amphetamine, sympathomimetic amines, opiates, opioids, etc.

How do drugs and other materials enter into saliva? One means is via active transport, as with

inorganic cations, calcium, and lithium. By far the most important mechanism of transfer from blood into saliva for the drugs of interest is diffusion. That diffusion depends on the lipophilicity of the drug and its degree of ionization, which is related to its pKa. The more lipophilic the drug, the more likely it is to transfer from the blood through the capillary membranes through the membranes into the saliva gland, itself, and into the saliva. By and large, the drugs that enter into saliva are unbound and unconjugated. Steroids and benzodiazepines, for instance, are highly protein bound, and thus, their concentrations in oral fluid can be quite low.

This figure illustrates the detection timelines of drugs in different matrices, including oral fluid, blood, and other matrices, based on a single drug dose. From our pain management work, we know that people are taking these drugs chronically, and we can detect them chronically.

Of particular consideration in using oral fluid as a drug testing matrix is the analytical sensitivity that we will require. Shown in this slide are the drugs of interest to us, including benzodiazepines and various opioids; the expected urine concentrations; the commonly used urine cutoffs; and then oral fluid cutoffs. For those people who are not familiar with the testing of oral fluid, the sensitivity needed to efficiently detect amphetamine and methamphetamine is about 10 to 20 times greater in oral fluid than in urine. For morphine, codeine, and the opioids the required sensitivity is about 10 fold greater. With cocaine, we are at two percent or less of the concentrations we would expect as cutoffs for urine. More importantly, THC is detected in oral fluid and, to a lesser extent, the THC acid in picogram concentrations, as opposed to nanograms. Obviously THC is not found in urine. Benzodiazepines, because they are so highly bound, very good sensitivity is needed to detect them at concentrations of one to two nanograms per mL.

The testing volume is an issue with oral fluid. We can typically collect greater than 30 mL of urine fairly easily. However, collecting more than two mL of oral fluid is difficult from a single collection. Thus, not only do we need much greater analytical sensitivity, but we also need to consider that we have a much smaller volume of specimen to work with.

In urine, we detect primarily the metabolites and sometimes the parent drug. Because of the transfer of drugs into oral fluids, we are primarily detecting the lipophilic component, which is the parent drug. In our pain management work, we also detect some metabolites that are fairly polar because these patients are taking high doses chronically.

Early on in the consideration of oral fluids, we were funded by NIST to determine if there was a predictable relationship between codeine concentrations between oral fluid and plasma. This was a clinical study in a clinical research center. Each subject received 30 mg of codeine. Subjects were instructed to brush their teeth and rinse their mouth prior to any collections. Blood was collected for harvesting of plasma and oral fluid was collected by spitting into an inert glass tube that had been previously silanized. 0.5 mL of oral fluid was analyzed by a GC/MS method with a reasonable sensitivity to five nanograms per mL, which is far less than the 40 cutoff that is being proposed. Reasonable analytical sensitivity is achievable for these drugs by GC/MS and certainly by GC/MS/MS and by LC/MS.

Our results are shown in this pharmacokinetic curve with oral fluid concentrations depicted in black and plasma concentrations in red. The x-axis represents time while the y-axis is a log scale of concentrations. In the initial time points, the saliva/oral fluid concentrations were much, much greater than the plasma concentrations; oral fluid concentrations were in the 4,000-5,000 nanograms per milliliter range while those in plasma were about 40. It was suspected that there was residual codeine in the buccal cavity of these subjects even though we had them brush their teeth, rinse their mouth,

etc. There is a plus and a minus here. The plus is the concentrations are very high, making it very easy to detect these drugs. The minus is that the relationship between the plasma concentration and the saliva concentration is very poor initially. It is not until two to four hours post dose that there is reasonable correlation based on the slope of those two lines, where we might be able to predict a plasma concentration from an oral fluid concentration. This table presents the same data in tabular form; this data include the collection time, the plasma concentration, the oral fluid concentration, and the ratio. At less than two hours post dose, the ratio is 300, 30, 13, etc. From the two hour to the four hour time periods, the ratio becomes reasonably predictable; the mean ratio from two to twelve hours is 3.7. In the first two hours after the collection, the oral fluid to plasma ratios are extremely high and not representative for predicting plasma concentrations. Two hours post dose, the buccal cavity is cleansed enough that we have a reasonably predictable concentration relationship.

Another result from this study is that stimulation of oral fluid causes an increase in the pH. We measured the pH of these oral fluid samples as we collected them. Using mathematical models that predict concentrations of these various drugs based on their pKa values and their percent of binding, we hoped to predict the plasma concentration from the oral fluid concentration. Shown here in blue is the theoretical ratio that we should have observed. Shown in orange is the actual ratio we calculated. At pH 7, which is right in the middle of the figure, the theoretical ratio of oral fluid to plasma was about two. Our measured ratio was 3.4. At pH 6, which is highlighted in red on the x-axis, the theoretical ratio was 20, and we measured 4.7. pH did not have the theoretical effect, at least in this study, on the disposition of codeine into oral fluid.

There were some reasonable conclusions from this study. One is we could collect oral fluid very simply though spitting. We could easily collect multiple specimens over time. Collection was very non-invasive and was under direct observation.

A limitation, obviously, was that for interpretive purposes, the oral fluid concentrations were not as predictive of the plasma concentration as we had hoped, specifically, early post dose when residual drug was in the buccal cavity. However, there was a reasonable prediction after a couple of hours post dose. Importantly, in terms of detection, oral fluid concentrations always exceeded those of plasma. We could detect the drug more readily and for a longer period of time. Unfortunately, pH did not have the theoretical effect that it was predicted to have.

Our results moved us to a second study. About this time, there were a number of collection devices being advocated for saliva/oral fluid collection. Shown in the far left is a device called a Salivette, which is basically a dental swab in a plastic container that is centrifuged and the oral fluid is collected at the bottom. Shown in the middle is a hooded collection device, which consists of a straw with an attached foam rubber pad that is placed in the mouth. Once the foam rubber fills with oral fluid, the straw is pulled back into this plastic housing and milked to remove the oral fluid from the device. Shown in the bottom left is a device called the finger collector, which worked much like a pacifier. The whole foam rubber pad was put in the mouth to collect the oral fluid. The pad is then milked. On the right are two of the more recent designs of collection devices - the Intercept and the Quantisal. These devices have pads to collect the oral fluid. Once the oral fluid is collected on the pad, it is placed in the tube that contains a buffer.

We designed a simple study to determine the effects of these various collection techniques on oral fluid concentrations. Five subjects were enrolled using the same protocol with codeine. The subjects were asked to spit, which was the collection protocol in the first study. Their oral fluid was either stimulated through chewing sugarless gum or stimulated acidically with a citric acid candy. Or the oral fluid was collected with the Salivette or the finger collector.

This slide shows the pharmacokinetic results with concentration on a log scale on the vertical axis and time on the horizontal axis. The real important information here is shown in the bolded numbers. The control is spitting and those concentrations are shown in black on the diagram. Notice that the control line is always higher than the other curves. For the finger collector, the mean concentrations were 77 percent of those collected by spitting. With acidic stimulation, shown in blue, results were only 28 percent, on average, of the concentration of the control. Those with non-acidic stimulation were, on average, 50 percent as high. So there is a pronounced affect from stimulating oral fluid on the concentration of at least codeine and perhaps many, many other drugs, as well.

This slide shows the same information in a different form, using the ratio of the collector concentration to control. The control concentration, circled in orange or brown, would have a ratio of one. At any time the concentration is above one would imply that the collection device concentration was greater than that of the control. Any time it is less than one would mean that collection had a lower concentration. The acidic ratio, which is noted in black, almost never reaches or goes over 0.5, meaning is has half the concentration of the control. The non-acidic stimulation, such as chewing, maybe reaches 75 percent of the control, but at all time points, it is much, much less.

The salient point is that collection technique does affect concentration. This slide shows the duration of detection or quantitation of codeine in these specimens in the second column. At the 12-hour time point, the limit of detection was one nanogram per milliliter while the limit of quantitation was five nanograms per milliliter. We asked how many could we detect as positive at 24 hours using one nanogram limit of detection. With the control, we were able to detect codeine in all of the samples at 12 hours. For the non-acidic stimulation, 60 percent were positive. With the acidic stimulation, only 20 percent were positive. How many of these samples were we able to quantify? With the control, over 80 percent were quantifiable. For these other collectors, less than half had quantifiable concentrations at 12 hours.

When we looked at whether we detect codeine a day after the dose, we found we still could in two thirds of the samples that were collected by spitting, but less than 40 percent with the Salivette, 20 percent with the finger collector, 40 percent if the oral fluid was stimulated by chewing, and none of the acidic stimulated specimens.

How long we could detect these drugs and how long we could quantify them is shown here in the conclusions for those studies and is related to dilution. Stimulation reduced the oral fluid concentration. Spitting was the most effective technique with concentrations three plus times greater than acidic and twice the non-acidic stimulations. Another conclusion that arises from this study is that literature on oral fluid needs to be reexamined to determine how the specimens were collected. There is no question that stimulating the oral fluid did affect these concentrations.

Another important factor was recovering the drug from these various collectors. Shown here are the hooded collector; the finger collector; an oral screen, which was very much like the hooded collector; the Salivette; and an early model of the Intercept. Shown here is the average recovery of drugs, including amphetamine, methamphetamine, etc., from these collectors. These drugs were actually collected at three different concentrations - low, medium, and high. Those data can be found in this study. Basically, the average recovery from the hooded collector for amphetamine was 28 percent, oral screen 28 percent, and Salivette 56 percent. The average recovery of amphetamine from these collectors was between 28 and 60 percent. Similar numbers were seen for methamphetamine and codeine. Morphine recovery ranged from 34 to 50 percent. Cocaine recovery ranged from 62 to 96 percent. Benzoylecgonine was the only drug that was reasonably recovered from all of the collectors

at above 90 percent. Interestingly, for PCP and THC, the concentrations or the percent recoveries were very, very low. My explanation for this, although it has not been validated, is that these are the two most lipophilic drugs in this list. Benzoylecgonine, which is much, much less lipophilic, has much better recovery. Almost all of these had very poor recovery for THC but reasonable recovery for the metabolite.

In a latter study performed in 2007, we included the Quantisal, which was a recent entry to the collector market. The different drugs tested, including benzodiazepine, methadone, and THC, had recoveries in the 90 percent plus range. The ranges for the other collectors are on the far right of the slide as background information. Of the other collectors, the lowest recovery for amphetamine was 16 percent while the highest was 57. The Quantisal had excellent recovery, even for THC at 80-90 percent. Recovery is an issue in terms of where we proceed with these guidelines. If we want to include collectors, we need to understand our ability to recover the drug from these guys.

The last issue is whether there is a validity measure for oral fluid collection. These data came from the previous studies in which we measured the IgG concentration from 100 oral fluid samples that we randomly selected from the total number we had. The mean concentration was roughly three with a standard deviation of 0.58. We asked six subjects to provide an oral fluid specimen to measure the IgG concentrations, which are marked with an asterisk. The mean concentration was 2.67. We then asked the subjects to rinse their mouths with 50 mL of water, and we immediately recollected. The mean concentration of the IgG decreased to 2.3 or 12 to 13 percent with rinsing. We had the same subjects do a second rinse of another 50 mL for a total of three plus ounces of water. The IgG concentration decreased to 2 micrograms per mL for a total decrease of 25 percent. Based on these results, I am not sure that IgG is a good measure of dilution. Obviously, this is a very small study and a single study. Proposed IgG cutoffs are 0.5 with current cutoffs of 0.1. I do not think a normal person will get down to 0.1. We need to consider whether IgG is a good indicator of dilution. Is there a good chemical marker for validity testing and, if so, at what concentration?

Topics for discussion include how is the specimen defined? Is it saliva? Is it oral fluid? Is it whole saliva? What is the volume of specimen because that drives the testing and the cutoffs. We need to decide if we will accept collection devices. If so, what are the criteria for percent recovery and volume that they collect? Will we allow spitting? I know there are some aesthetic adversities to this, but in the subjects that I have dealt with, they have had no problem with it. What cutoffs should we set? What drugs and metabolites will we test for? This is important, too, in terms of feeding back to the volume of the specimen that we can collect and our method of collection. Is there a reasonable measure of specimen validity? Can we measure the volume collected? Can we measure the weight collected? Certainly, it would be nice to have a chemical marker that would tell us we have a valid specimen. We need to also consider the effects of stimulation.

Oral fluid has a lot of advantages. I hope we do not get bogged down in the negative aspects. One advantage, for example, is that we can do these observed collections. There are no special facilities or requirements for the collection site. There is a potential relationship to blood, which is helpful in a number of arenas outside of the workplace. These specimens are very easily transported because of their small volume and their storage in plastic tubes. Oral fluids are easily analyzed. Because oral fluid is 98 plus percent water, we do not have many of the matrix problems we have with blood or even urine. Certainly, if we have more than one specimen collected from a donor, it is very complementary information to what we can obtain with urine, hair, sweat patches, and others.

Thank you very much for your attention. Janine, do we have questions?

Dr. Cook: Do any members of the Board have questions for Denny? I want to introduce Dr. Marilyn Huestis, who will be speaking to you about drug analytes and cutoffs as related to oral fluid.

Drug Analytes/Cutoffs

Dr. Huestis: Good morning. I am very happy to be here. We have been doing research on oral fluid, beginning with Dr. Ed Cone before me with whom I started my doctoral work, so we have lots of data. Because I only have a limited amount of time, I will point out some of the most important factors on each slide. I will be happy to answer questions later. Denny has presented some similar information, so I will go quickly over the duplicate information.

Different cutoffs have been proposed for oral fluid, including the proposed SAMHSA cutoffs from the 2004 publication in the Federal Register; the DRUID cutoffs, which is the new driving under the influence of drugs program in the EU; and Tailloires Expert Group, which Mike Walsh, myself, and others were a part of. DRUID has conducted tremendous numbers tests, and they have standardized testing across Europe for the DRUID program. There is our Controlled Drug Administration research data that I think will guide some scientific decisions. It is very nice to have a scientific database on which to base your policy decisions.

We have a very exciting smoked cannabis study that is currently in progress. To date, we have enrolled four subjects. This study will provide tremendous data for the Board to consider. Subjects are smoking a 6.8 percent THC cigarette, which is within the range of what is typical for the U.S. This is double what we have ever dosed before. The key feature of this study is that we are analyzing all of the samples in real time – the whole blood, plasma, and the different oral fluid devices are analyzed immediately upon collection. We are also performing a one-year stability study. This study results will provide a lot of information, including what additional data and research we will need. This study includes some things that we have learned in our many years of working with this.

Denny discussed some potential advantages of oral fluid. I think one key thing I have not heard said yet today is that identical results will not be obtained from the different matrices. Each matrix provides unique information, and we should not expect to get exactly the same number of positives with any different matrix. That is important for us to consider. What is very nice about oral fluid is that it is a less invasive collection and a direct observation collection, so adulteration may be less of a problem. Denny mentioned that you do not need a same-sex collector or a specialized facility. Also, I would like to stress that the detection window is completely driven by the cutoff that is selected. Depending upon the selected cutoff, a different number of positives is obtained.

Some other factors that affect drug concentrations are the physiochemical characteristics of the drug, the specimen matrix, and the route of drug administration, which is important also, not only on concentrations, but what analytes you might expect to be present. Denny mentioned how the pH of oral fluid changes, and that is a very important factor. The impact of the amount of drug is unknown. Can you overwhelm the system? If you have really high concentrations of drug in the blood, will we get the same percentage of drug in oral fluid? Those studies are hard to do, especially in a controlled situation where we cannot administer such high doses.

The physiological factors are really important. All of the work that I will present today was done in authentic oral fluid. We use specimens collected from donors in the lab for our calibrators and controls. We have a number of individuals in the lab we cannot use their saliva specimens because they have such thick, mucus saliva.

The time factors are very important - what analytes are expected to be seen at what time points after drug use. The specimen collection variability is very, very important. An area in which we require much more information is in the stability of the different analytes in the oral fluid specimens. Of course, just like any other biological matrix, there is within and between individual variability.

Denny discussed passive diffusion as being the primary method for drugs to enter oral fluid; active secretion is an entry mode for only a few substances. What is really important is the fact that contamination of the oral fluid cavity can occur, especially if drugs are insufflated or smoked. We found, very interestingly, that many oral medications do not contaminate the cavity if they are formulated in some type of capsule or with an enteric coating. We have had a number of oral controlled dosing studies where we do not have contamination. We collect the first samples later when it gets into blood and then into the oral fluid. There is the question of whether or not, if someone was in an environment where people using/smoking the drug, you would have passive inhalation.

There are a number of different collection methods. In medicine, they use passive drool, which is collected in a tube by a process that takes forever. The flow of fluid is not stimulated in any way. I think that passive drool is totally unreasonable to be considered here. Expectoration or spitting is collected using either non-stimulated devices or stimulated devices that have citric acid present.

Really, really key is the elution solvent. What solvent is used to remove the drugs from the pad and into the matrix that you will use in testing? That is extremely important factor. The manufacturers have come a long, long way from the first generation of devices, which did not elute appropriately and thus produced low recoveries.

Lipophilicity is important, but we have not followed it up yet in our studies. Christine Moore published that she found carboxy glucuronide in oral fluid. We have not followed up on that to see whether or not we could measure it.

The pKa of the drugs, as Denny mentioned, is important and related to the term ion trapping. Drugs, both the non-bound and the non-ionized forms, cross from the blood into the oral fluid. Because of the higher pH of blood and the lower pH of oral fluid, neutral molecules, crossing over the membrane from the blood into the oral fluid, will ionize at the lower pH. This process drives the drugs across the membrane, creating an equilibrium between free drug, non-ionized known as ion trapping. Any basic drug will be at a higher concentration in oral fluid than in plasma because it is ion trapped into that fluid. And, of course, the molecular size of the drug is important as to how well it can transverse those membranes.

Denny had great data to demonstrate the effects of chemical or mechanical stimulation. Similarly, we found in our studies that the amount of oral fluid that can be produced can be increased tremendously by stimulation. This is a much bigger factor than the pH changes that occur with the bicarbonate increase. If the flow is increased, the concentrations will decrease.

The volume of oral fluid that is collected is very important. I will tell you some very surprising things. We know that stimulants cause reduced salivary flow. Just like the comment this morning on shy bladder, there is dry mouth. In our smoked cannabis study, devices were held in the mouth for as long as fifteen minutes without the device color change because the subject did not produce sufficient oral fluid. Soon after cannabis smoking, and probably after the ingestion of methamphetamine and other stimulant-type drugs, such as maybe cocaine, you can expect that it will be difficult to collect a specimen, even within 30 minutes. You will collect less specimen, and it will be very thick. These donors have a difficult time even providing expectorated specimens.

In a wonderful study out of Finland on the effects of the elution solvent, they tested devices in which they did not leave the pad from the collection device in the elution solvent for very long. Many of the manufacturers designed the devices on the premise that the collected specimen would be in contact with the elution buffer for at least 24 hours. This time period reflects the transit time for the device to reach the laboratory for analysis. If the pad does not stay long enough in the elution solvent, recoveries are reduced. For people to use the devices appropriately, the manufacturers should list the minimum contact time for their stated recoveries. This is very critical, and I am a big proponent of manufacturers publishing this and including it, along with recoveries, in their package inserts for every lot. Because this is too much to ask every single laboratory to do that, the manufacturers should provide that information. Certainly, there are some limitations. Oral fluid has a smaller volume, but if you are using a device, the elution buffer increases the sample volume. There is still an issue about analytical sensitivity. One of the biggest problems is removing the drug from the pad of the collection device. Another important issue is the volume. The volume of oral fluid collected, as well as the volume of the elution buffer, must be measured with very high precision.

One issue with elution buffers is whether or not the sample can proceed directly to analysis, for instance, LC/MS/MS. Is a screening method required? The elution buffers may interfere with LC/MS techniques, producing matrix effects. We have found in our work, as have other researchers, that the matrix effect issue is real and either a liquid-liquid or a solid phase extraction is required to eliminate some of that matrix effect. We also have to consider the passive contamination issue.

For amphetamines, the SAMHSA screening cutoff is 50 nanograms per mL. One question is whether or not the screen will adequately cross-react with MDMA and perhaps MDA or whether a separate target screen for those analytes is needed. The confirmation cutoff is 50 nanograms per mL for each of those analytes. Right now, the Guidelines require amphetamines to also be present in a methamphetamine-positive specimen at concentrations greater than the LOQ. I will present data on that will cause samples to be missed, if that is required. The DRUID confirmation is half of that at 25 nanograms per mL. As for urine testing, the program has always required that laboratories perform at 40 percent of the cutoff. At about 10 nanograms, the labs would need to perform with good sensitivity, specificity, and precision. This is very doable, as you will see. The Tailloires confirmation cutoffs were recommended at 20 nanograms per mL. SAMHSA is more than twice what the others require. A number of countries have legislation already in place for oral fluid testing at the roadside and in other areas. The Belgian legislation dictates a cutoff of 50 nanograms per mL for screening and 25 nanograms per mL for confirmation. They only require amphetamine and MDMA testing. The French legislation is at 50, similar to what SAMHSA is for those three analytes. Australia initiated drugged driving legislation in 2003 and has been very successful at reducing it in that country. The Australian process, which I don't think we could not do here in this country, involves two point-of-collection devices, the Cozart and the Drug Wipe test. The individual's oral fluid is tested by two different devices. Both of these devices are older models with many limitations, resulting in many false negatives, rather than false positives. If a positive result for methamphetamine, MDMA, or THC is obtained on either device, they confirm all three analytes. Many and most of the positive cannabinoid tests come from the positive methamphetamine or MDMA and not from having a positive cannabinoid screen. This is not a policy that we have in place here at all, but it is real important to remember.

Methamphetamine is a basic drug, which implies ion trapping and higher concentrations, most likely. The half-life of methamphetamine is very pH-dependent, which will really affect the half-life of the drug in oral fluid. This is really interesting data. Here we have plasma methamphetamine and amphetamine after a single 10 or 20 milligram extended-release dose. Notice is that the high

methamphetamine is about 30 nanograms per mL in plasma. Here is the low dose and the amphetamine is a much smaller proportion of the methamphetamine. Here are the data on oral fluids. Compared to the 30 nanograms per mL in plasma, the high peak in oral fluid is almost 150 nanograms per mL. It will be at much higher concentrations in oral fluid, and thus easier to detect, with a similar or a little bit longer detection time. In oral fluid there are much higher Cmaxs compared to plasma. The detection times of the low and the high doses are much lower than urine, implying much shorter detection times in oral fluid. This shows when the peak concentration occurs after these doses. I want to point out that our methamphetamine doses are certainly less than someone who is abusing methamphetamine by the IV or smoked routes, which would deliver higher doses. The Tmax for plasma and oral fluid is about five hours, where the Tmax in urine is about 15 hours, which causes differences in detection windows. If someone uses multiple doses, such four daily doses in a row, there is some build up in oral fluid even after only daily doses once every 24 hours. The more they use, the higher the concentrations will be. These are the 24-hour detection rates for specimens collected over a 24-hour period. These are the detection rates at different cutoffs. Notice the sensitivity that we need is really not difficult to obtain. Our limited quantification was 2.5 nanograms per mL. At this cutoff, 60 percent of the samples were positive within 24 hours. Using the SAMHSA cutoff, positivity drops more than half to 23 percent. This is one of the reasons I think that the DRUID program and Tailloires suggested using cutoffs lower than 50 nanograms per mL. If you include the amphetamine at the limit of quantification, it drops another five percent. But there are many false negatives occurring at the early time points because it takes longer for the amphetamine to enter into the system than the methamphetamine. Also, at the end time points, amphetamine had lower concentrations. You will miss samples at the beginning and at the end of the time course, if you require the amphetamine. Methamphetamine and amphetamine are certainly the important analytes of interest. One thing to consider is whether to lower to 25 to improve sensitivity. That means that we need to do a good job at 10, which I think is very doable. My suggestion would be to not require amphetamine at the LOQ. We have data on how many true positive specimens that would eliminate.

Switching to MDMA and its metabolites. In this study, we used both expectorated oral fluid and oral fluid collected with the Intercept. We enrolled 30 subjects who all had a history of MDMA use. The subjects resided on the closed unit for the study, so no other drug use was possible. Very importantly, the dosing reflected doses used at the recreational level. These high dose data represent what would be found in the field. We were limited to 150 mg maximum for safety purposes. There was one subject that exceeded that amount based on his weight. There were seven days between dosing. Oral fluid was collected by non-stimulated expectoration, but remember, the process of expectorating has some stimulation associated with it. Oral fluid specimens were collected for up to one week.

The metabolism of MDMA has this minor route from MDMA to MDA. The major route is an O-demethylation, which is a product of HHMA that is not quite stable. This analyte is not commercially available, but we were able to obtain some that was synthesized for us. This analyte is extremely difficult to measure, so I absolutely do not think the two dihydroxys should be considered as biomarkers. The HMMA and the HMA are very important compounds in urinary excretion, along with the glucuronides and sulfates. We wanted to analyze all of these analytes, so the analysis was performed in oral fluid without hydrolysis. We collected 503 oral fluid specimens from 27 subjects. Listed here is the dose range. A typical recreational dose is about 75 milligrams in a tablet. We had 576 from the high doses and 210 from placebo. We analyzed those to determine how long to extend our detection window. We did have some subjects withdraw after only receiving one dose. Here is the total number of samples in this group. We had 875 specimens, or 68 percent, positive at the LOQ, which was five nanograms per mL. There was a huge range of concentrations. Most of those were positive for MDA, as well. Positivity dropped with the 25 DRUID cutoff to 58 percent and with the SAMHSA to 55 percent. In this study, there is not much difference between the 50 and the 25 cutoffs.

That does not mean that it would not be different in a different population. In this population of true positives, we missed almost 45 percent. A much lower percentage will be positive for MDA. Again, I absolutely would not recommend requiring the presence of MDA to confirm an MDMA because many positives specimens would be missed.

With an oral dose, the drug is first detected in less than an hour, even at the SAMHSA cutoff. Shown here are the ranges and the detection times at these cutoffs. Notice, this lists only those exceeding cutoff. At the 50 microgram per liter or nanogram per mL, there were a large number that did not have any positive samples that we couldn't determine the time of detection, but it is a quite short time window.

With the Intercept collection, this line across here represents the SAMHSA cutoff, the DRUID cutoff, the Tailloires cutoff, and the limit of quantification at the one to three dilution. This is shown on a log scale. Also shown are the hours and the low and the high doses. The actual time course is depicted. With whatever cutoffs are being considered, we can reanalyze the data since our LOQs are low. This is just showing an inset early in the time course.

MDA is much more a minor metabolite, but of course, MDA can be abused itself. We do not have a lot of that in this country. Many of the MDA specimens will not be picked up at those doses when it is a metabolite of MDMA. Depicted here is the duration of detection in oral fluid for the last detection. After the low dose, the last detection is about one day, and after the high dose, detection is up to two days. Very little MDA is be detected at the 50 cutoff; with the high dose, last detection is about eight hours.

This graph takes some time to get used to, but it is really a nice way of looking at the data. We start with the SAMHSA cutoff, shown in blue, because it is the highest. So if you see blue, it means that it was detectable at all of the cutoffs. If you see the stripes, it means it was not detectable at the SAMHSA cutoff, but it was detectable with DRUID. If you see the yellow, it means it was only detectable at the LOQ of five nanograms per mL. This graph allows you can see when you will begin to detect the drug and what percentage of the specimens is positive. This is followed by a long period of time where the drugs are detectable at all of the cutoffs. During withdrawal, you can determine how long the drug is detectable at the different cutoff levels. This is really a great way to determine what cutoffs you want. The MDA rates are shown here. Again, at the SAMHSA cutoff, you will not detect it for much of a length of time.

These are realistic MDMA doses. The correct analytes are the ones that you should be analyzing for. We found no HMMA and no HMA, and thus, they should not be analyzed. That is an important thing because they are very important in urine.

One thing to consider is whether or not you want to go lower. If you require the presence of MDA, you will miss a large number of the specimens. MDA was never found alone; whenever MDA was present, MDMA was also present throughout the entire time course.

Switching to opiates. The SAMHSA requirements are screen and confirm for morphine and codeine at 40 nanograms per mL and 6-acetylmorphine at 4 nanograms per mL. DRUID is at half that at 20 nanograms per mL, and they also include methadone. They use 5 ng/mL on the 6-acetylmorphine. The purpose of the Tailloires confirmation cutoffs was to standardize cutoffs so that studies could be compared. Because there are many studies out there using different cutoff concentrations, the data could not be compared. These were recommended cutoffs for driving under the influence of drugs studies. Belgian legislation screens and confirms morphine at 10 nanograms per mL. They do not

analyze for codeine. They use 5 ng/mL for 6-acetylmorphine. The French legislation is for morphine and 6-acetylmorphine at 10 nanograms per mL. Australia only includes MDMA, methamphetamine, and THC.

In this controlled codeine administration study, subjects received 60 or 120 milligrams, based on a 70-kilogram person, so the actual dosage could go up or down a little based on their weight. We collected specimens at baseline, as well as for 72 hours afterwards, for a total of 1200 samples. We used three different collection devices. In this older 1990 study, we used citric acid candy for stimulation and expectoration for collection. We found out quickly that if you stimulate the flow, the drug concentrations are reduced. We compared the candy results to the Salivette neutral cotton swabs and the Salivette citric acid swabs.

Because codeine is a base, ion trapping is a factor. The plasma concentrations and the oral fluid concentrations are shown here after the low and the high dose. Much higher concentrations are found in oral fluid than in plasma with a basic drug and ion trapping. Listed here are the detection times. For oral fluid at the limit of quantification of the assay, the detection time is a little less than one day. Using the SAMHSA 40 nanogram per mL cutoff with the low and the high dose, detection time is about seven hours.

Another analyte to consider for codeine is norcodeine, a metabolite. The body, when metabolizing drugs, tries to make them more water soluble and more polar so that it can excrete them more efficiently. Because norcodeine is more polar, you would expect that the concentration of norcodeine in oral fluid is less than in plasma. Again, that equilibrium across the plasma membrane is important. Notice that the proportion of norcodeine to codeine changes dramatically during the time course because norcodeine is a metabolite and it sticks around longer. Thus, we should consider norcodeine as an analyte, as well.

26.5 percent of the specimens were positive for codeine at these therapeutic doses. A wide range of concentrations was found over the three days, as you would expect. Only 13.7 percent were positive for norcodeine, which always present with codeine. Thus, adding norcodeine would not increase your detection rate whatsoever. Neither morphine nor normorphine were detected at these doses in oral fluid. Notice that we started to detect the drugs very rapidly post dose. The codeine was given orally in a capsule. The Cmax occured within four hours. Here are the concentrations -- 642 nanograms per mL and almost 1,600 nanograms per mL. Yet, the detection window was very short at seven hours. This totally different study examined opioid prevalence in opioid-dependent pregnant women on methadone treatment. The presence of opiates in this study represents relapse to heroin. This group had a very high relapse rate for 50 percent. We used an oral fluid method with very low detection rates. These women produced oral fluid specimens three times a week on Monday, Wednesday, and Friday throughout pregnancy so we would be able to detect relapse. Also collected was urine that was analyzed non-hydrolyzed urine. The data would look different than the urine was hydrolyzed. 6-acetylmorphine was present in 82 percent of the opioid-positive specimens. The median was only five nanograms per mL with a max of 434 nanograms per mL. 50 percent of the non-hydrolyzed urine, at a really low detection limit, was positive for 6-acetylmorphine. This percentage is much higher than we normally see. This use is heroin use. The median concentration was 43 nanograms per mL with a maximum of 600 ng/mL. We measured heroin in oral fluid and found 42 percent of the positives were positive for heroin, as well, at two nanograms per mL. We could not measure that in urine because it would be hydrolyzed very rapidly. Codeine was present in low concentrations. Norcodeine was present in 25 percent of the samples at only three. Morphine was present in 24 percent of the specimens at a very low rate of eight nanograms per mL. 6-acetylcodeine, another biomarker for heroin, was also present. This study is a real life situation of heroin abuse. Notice the wide variety of

markers of heroin use that are not found in significant concentrations in urine. Again, sensitivity has to be really high.

Because it is a basic drug, oral fluid S/P ratios are greater than one. You are able to detect heroin, 6-AM, or acetylcodeine to clearly identify heroin usage, which is an important advantage and would certainly help the MROs in their understanding and reporting of results. Lance Presley, using a low three nanograms per mL cutoff for 6-acetylmorphine, found that 67 percent of the opiate positive workplace tests were positive for 6-AM.

There is only one small study in the literature published by Tim Rohrig, in which subjects ate poppy seeds. Positive results for morphine were obtained for up to one hour post ingestion using the 40 nanogram per mL cutoff. If the poppy seed story does not go away, a waiting period of some time may be required. Certainly, this study should be repeated because this is important research that will make an important contribution.

Switching to cocaine and benzoylecgonine. The SAMHSA requirements are screening at 20 and confirming at eight. The DRUID uses ten, a very similar confirmation cutoff. Most of the DRUID laboratories do not perform an immunoassay first; instead they proceed directly to LC/MS/MS. They identify all of the positive specimens at the confirmation rate. That is very important because you can screen up to 28/29 of these compounds in a single oral fluid sample. Since the oral fluid volume is limited, they have found this protocol to be very effective. Whether this protocol could be done on a huge scale, I do not know, but that would certainly be an issue. The Tailloires recommended either analyte at 10 nanograms per mL. Belgian legislation screens at 20 and confirms at 10. French legislation just requires the confirmation at 10. It is not included in the Australian legislation.

This is a controlled cocaine administration study involving two doses - the low dose of 75 milligrams based on a 70-kilogram individual and the high dose of 120. A typical line of snorted cocaine is about 100 milligrams. Individuals who use cocaine can become tolerant and use huge quantities, either by IV or smoking, as well. This study was done by subcutaneous administration based on safety requirements at that particular time. Very good doses are obtained following subcutaneous administration. We examined three different collection methods in this older study. Here is what is very interesting in the time courses. We analyzed cocaine, benzoylecgonine, and ecgonine methyl ester, to consider whether ecgonine methyl ester might also be a good analyte to look at. The peak concentrations, shown in nanograms per mL on a logarithmic scale, cross the different cutoffs, 20 and 10, right here. At the cutoffs, a lot of benzoylecgonine is present, not as much of the cocaine, and a very similar amount of ecgonine methyl ester and other metabolites. These are the percentage of positives, and these are positives by expectoration following citric acid candy. 55 percent were positive for BE, 47 percent for EME, and 35 percent for cocaine. This is interesting because cocaine is a base, and thus ion trapped into oral fluid, but a lot of benzoylecgonine is present in oral fluid. Whether it is crossing over from the blood or whether some kind of spontaneous hydrolysis to benzoylecgonine is occurring in the oral fluid, I am not aware of any research that has been able to shed any light on that. It is clear that benzoylecgonine is an important analyte to include. Here are the peak concentrations present for each of the different methods of collection. The Salivette device is a cotton roll that is placed in the mouth and uses no elution solvent. We learned the hard way that the pad absorbs the drug, and the drug does not come off easily. The use of an organic solvent to elude the drug also dissolves the pad itself. Though it is difficult to remove drugs off of that device, notice that in every case, the concentrations are higher than what was obtained with expectoration. I think you see where I am going with this. We have found expectoration not to be the best sample for two reasons. In general, it is not as sensitive. There are many more problems with the extraction. The mucus that is present in the specimen and your ability to extract the drug out of the expectorant are

issues. Also, donors do not like to do it, as well. We found over multiple drug classes that the concentrations tend to be lower.

The concentrations of EME are very similar to the concentrations of benzoylecgonine. Here is the initial detection with its very rapid appearance in oral fluid. Remember, administration was subcutaneous, so the drug had to be injected, enter into the blood, and then enter into the oral fluid. The maximum concentration occurred at about three hours. Notice that the BE Cmax is significantly later than the cocaine Cmax at eight nanograms per mL. Notice that the detection time with benzoylecgonine is about one day, and it is twice as long as for cocaine. Here are the detection times at the limit of quantification for the low and high doses. Displaying the data in the same way, notice at the DRUID cutoff of ten, cocaine is detected in oral fluid because of the ion trapping for about 12 hours for the low dose and 24 hours with the high dose. The only difference is right here; not much is gained with the eight nanograms per mL cutoff over the DRUID cutoff.

Here is benzoylecgonine. Notice the differences between cocaine and benzoylecgonine. Cocaine is important when the drug has just been used while benzoylecgonine provides a longer detection window. The entire spectrum is better covered by having both of those analytes present. The benzoylecgonine to cocaine ratios change over time because the metabolite is formed and has a longer half-life in oral fluid as well as in plasma.

Referring back to the opioid-dependent pregnant women study and comparing oral fluid to un-hydrolyzed urine, 19 percent of the oral fluid samples were positive, while only four percent were positive in urine. That difference is because there was not a hydrolysis. It shows that 70 percent were positive for cocaine, 66 percent for benzoylecgonine, and dropping a lot to ecgonine methyl ester.

There are some other older studies showing that cocaine appears rapidly in oral fluid after IV, injection, smoking, and intranasal administration. Cocaine is stabilized in the more acidic pH of oral fluid. Though not fully stable, it is more stable than in plasma. The BE ratio increases to greater than one in about four to six hours.

For the potential analytes, we found adding ecgonine methyl ester only identified three additional positive specimens. Thus, it is probably not necessary to include EME. Every time cocaethylene was present, it was also cocaine or benzoylecgonine. Using the 10/10 nanogram per mL cutoff, which is the DRUID cutoff, you had 26 percent better specificity while only reducing the sensitivity by 6.8 percent. Whether you are at the eight or ten cutoff, you are pretty close. Compared to the LOQ at 2.5 nanograms per mL, you do miss 112 positives, but you eliminate 183 unconfirmed positives. I think that is important. The efficiency was greater than 80 percent.

Switching to cannabinoids. Currently, the SAMHSA proposed cutoff requirements for THC are four nanograms per mL screen and two nanograms per mL confirm. DRUID uses a one nanogram per mL confirmation. Tailloires also recommends one nanogram per mL. The Belgian legislation is interesting. It is very high because they could not find a device that worked well at lower levels. So they have written the law so that if the devices become better and more sensitive that they can lower their limits. They expect to reduce this, but they are currently 25 for screen and 10 for confirmation. The French are at 15. The Australian legislation requires screening with both devices with two nanograms per mL for their confirmation. They will confirm for THC on a methamphetamine positive specimen.

This data is from a large study we did looking at tolerance and antagonist-elicited withdrawal. We enrolled heavy, chronic cannabis users who were housed on the unit. The subjects were given 20

milligrams, which is a whopping oral dose. They received 37 doses of up to 120 milligrams per day of THC for eight days. We collected 440 oral fluid specimens using the Quantisal device and through expectoration. We were able to examine many different things in this study. Because several subjects were smoking just prior to entering the unit, we could examine cannabinoids from self-administered smoked drugs. Because of the study design, we could study single dose oral pharmacokinetics, followed by the study of continuous dosing, and then finally study detection times once we stopped administering the drug. I know there is a lot of interest in carboxy-THC as a potential analyte. We have some very interesting data on that. To analyze for carboxy-THC took two years of method development. We used one mL of the oral fluid/Quantisal buffer mixture that is at one to three dilution, representing 0.25 mL of neat oral fluid. Cold acetonitrile followed by centrifugation is used to precipitate and separate out the proteins. The supernatant undergoes a solid phase extraction. The first eluent contains all the neutral cannabanoids - THC, cannabidiol, cannabinol, and 11-hydroxy-THC. The carboxy-THC is derived from the second elution off the solid phase extraction. We have two different GC/MS instruments - a two dimensional GC/MS with cryofocusing, which means its cost effective. In the first one, we use electron impact and obtain THC. 11-hydroxy is never found at the LOQ of 0.5 nanograms per mL; I am sure it's there, but at picogram per mL concentrations. The second eluent goes to a different GC/MS with negative chemical ionization at a sensitivity of 7.5 picograms per mL. I think a ten would be very achievable in most labs. Notice the different LOQs. THC, CBD, CBN, and 11-hydroxy are all at 0.5 or 1.0 nanograms per mL. The carboxy-THC is at 7.5 picograms per mL. What we were so surprised at is that only 20 percent of the samples were positive for THC, while 98 percent of the samples were positive for carboxy. These are the median and the maximum concentrations.

This median represents self-administered smoked cannabis at entry. The data range from none detected all the way up to 400 nanograms per mL. There are 19 to 20 hours before the first dosing. Notice that the THC starts decreasing and the wide ranges.

This is after the first 20 milligram THC dose. The median is still going down. For the two days of 100 milligrams THC per day orally, the median was zero with one or two positives. From the fifth to the seventh day when subjects are receiving 120 milligrams per day around the clock, the median is zero with one sample at 1.1. After the last dose, none is detected.

Examining carboxy-THC, at entry, the median is about 21 picograms per mL, with a wide concentration range. The positives begin pre-dose if somebody has smoked recently; the oral fluid levels rise even though they are on a closed unit. The carboxy-THC will peak at a later time point. After the first oral dose, carboxy-THC starts to increase and continues to increase. Here are the concentrations. Our peak was over 1,000 picograms per mL. After the last dose, it remains for quite a long period of time. This is what the carboxy-THC data looks like, with plasma data shown in green and oral fluid in blue. The numbers represent the number of doses between those oral fluid samples. It just goes up, and then after the last dose, it slowly drops off.

In plasma, THC starts to go down and is present at low concentrations. After the first dose, a bump is noticeable followed by a decrease. THC is very rapidly metabolized to the carboxy-THC and that is what is detected. In plasma, the carboxy-THC rises after the first oral dose; oral fluid mirrors this trend.

There is not much data on duplicate specimens. We had eight sets of times where we collected two specimens. The devices were in the mouth at the same time collecting the specimens. There was really good duplication for the two specimens which is an important finding for split specimens.

In the Niedbala study from 2001, there was very good agreement with specimens that were simultaneously collected with the Intercept device on the left and the right side of the mouth. He found, doing confirmation of THC, the last positive oral fluid test after about a 22 milligram THC cigarette of about 34 hours.

In an earlier study where we administered up to 14.8 milligrams oral THC in hemp oil and other products, we found no positive oral fluid specimens. If the person is taking THC orally, THC is not detectable.

Day et al. and then Moore et al. were the first to report carboxy-THC in oral fluid. I think it is an important analyte because it hopefully obviates the problem of passive contamination because it has to be taken into the system and metabolized. It is not present in cannabis smoke.

We do not have a study that examines passive exposure and whether passive exposure would produce enough carboxy-THC to be positive. Niedbala et al. have two studies. The first study was inside a closed, small van where many people were smoking, and the devices were inside the van. The devices yielded positive results with the passive smokers. The concern was whether the devices contaminated. The study was repeated with the devices stored outside the van. The subjects were exposed to the same levels of smoke. The oral fluid specimens were collected outside of the van, and this time, the subjects were not positive. Personally, I do not think the carboxy-THC will appear from the small amount that you would obtain in a passive situation, but we do not have the proof.

These are the concentrations we obtained in our older study - up to 5,800 nanograms per mL of THC twelve minutes after cannabis smoking. Notice how rapidly it drops to 81 micrograms per liter within 20 minutes. Gerald Kauert in Germany found concentrations of 900 and 1000 nanograms per mL of THC 15 minutes after smoking decent concentrations of THC. He found it drop to 18 nanograms per mL in six hours.

Some things to think about for cannabinoids in oral fluid. Do you screen for THC and then confirm for both? Do you screen for THC, confirm for THC, and then only utilize carboxy-THC in difficult challenge cases? If you do that, then every positive will probably be challenged. Do you screen for carboxy-THC and confirm for carboxy-THC? There are immunoassay reagents out there for doing carboxy-THC in hair that are sensitive enough to pick it up in oral fluid.

The highest recommended cutoffs are four for the screen and two for the confirmation, but you could go lower like they are doing in DRUID or as Tailloires recommended.

Absolutely, expectoration is not the specimen of choice. In our new study where people are smoking and we are analyzing the samples in real time, only four subjects have enrolled and completed the study. This study will conclude in mid-March. We have found lower concentrations in the expectorated samples than we do in the device samples. Also, our first week of stability studies shows that we are losing more than half of the THC in expectorated samples. For a device, the eluent not only helps remove the drug from the pad, but it also stabilizes drugs in the eluent.

How long should the waiting period be to ensure that there is not passive exposure? It certainly might be possible for workplace and treatment programs, but for DUID testing that would absolutely be very difficult to do.

I believe that you need the diluent and preservative in the collection device, not only to recover the THC, but to stabilize it as well. We have to be concerned about people not doing matrix interference

studies and not extracting the drug to get rid of matrix interferences.

We have very little data, as Denny said, for an oral fluid biomarker. Because it is an observed collection, is it necessary? I was very interested to see Denny's data. But one finding for cannabis from DRUID is that rinsing the mouth really reduces the detection of cannabis because smoking contaminates the mouth and the tongue. Washing or rinsing your mouth can really lower your detection of cannabis and other drugs that might be smoked.

In a current study, we are examining expectorated oral fluid; the Quantisal device; and the Drager drug test, which is an onsite drug test; urine; whole blood; and plasma. We are also performing a full stability study. In the second part of the study, we will be looking at the Intercept, the StatSure device, and the Drager 5000, which is an onsite test that has a way to collect a sample that could then go to the laboratory for confirmation. So far, we have found major THC losses within one week of refrigerated storage for expectoration. A major problem we have found is that the cannabis-smoking subjects have difficulty producing enough oral fluid for the devices to indicate complete specimen collection, even when the devices are left in the mouth for 15 minutes. We have had big problems with these subjects being able to expectorate. In the first half hour, there are many issues with obtaining enough oral fluid volume.

The other issue is that any kind of stimulant will cause the production of very mucus saliva. This specimen must be centrifuged, but if you spin the expectorant, some of the drugs that bind to particulate matter in the oral fluid are lost in the precipitate. I think that is part of where you are losing the drug. When you place those samples, even though diluted with water, on solid phase extraction columns, much of the drug passes right through. The mucus does not allow a good extraction of the drug onto the solid phase extraction column.

Although PCP can be measured in oral fluid, there are no controlled studies and there never will be. My recommendation is expectoration is not the best specimen for many reasons. PCP is not included in the DRUID, Belgian, French, or Australian programs.

There is the RTI oral fluid proficiency program that showed at 15 nanograms per mL labs could perform very well, but there was a wide range. There is a report in the literature of an authentic PCP study involving the administration of 38 ng/mL, though was not a controlled administration. We do not know anything about time.

I want to thank all of the people in my group, who helped do all of this work. Thank you very much.

Dr. Cook: If anybody has questions for Marilyn, please ask her at break. We will take a break now and reconvene at 11:00 am. We are behind schedule, but we will continue until we are finished.

(Break)

Dr. Cook: I will introduce Dr. Frank Esposito, who will be discussing the various methodologies related to the oral fluid specimen for drug testing.

Methodologies (Collection Devices, Screening Immunoassays, Confirmatory Tests)

Dr. Esposito: Thank you and good morning. This presentation will provide a general overview of the methodologies used for oral fluid collection devices, screening immunoassays, and confirmatory tests.

Let’s begin with some of the types of oral fluid collection devices on the market. There is the neat oral fluid collection device. There is a passive pad that is simply placed in the mouth of the donor with a timed collection or with a volume indicator on the handle. There is the chewable pad, with or without impregnated compound to stimulate oral fluid secretion. There is active swabbing with a pad, with and without a volume indicator. And there are other collection devices, including an oral cavity rinse.

Let’s examine several methods for collecting oral fluid. One method is the collection of neat oral fluid by expectoration or spitting. This is a photo of such a device that consists of a funnel that is screwed on a glass container. The donor simply spits into the container until the required volume is attained. The advantages of this method include a large volume can be collected. Most of the time, the collection time is short. No mathematical conversion of the quantitated drug to a neat value is required. There should be complete recovery of the drug from the device.

The disadvantages of this method include it can be unsanitary and biohazardous. It is subject to bacteria and yeast contamination. It is difficult to manipulate the specimen to provide a split specimen or a laboratory aliquot for testing.

Here is an example of one of several collection devices with an absorbent pad. The device contains a cellulose collection pad, which is placed in the donor’s mouth. The pad becomes saturated with oral fluid and is removed either at the end of a timed collection, or with some devices, when the volume indicator dye moves up to the window on the handle. The collection pad is placed in a transport tube that contains a liquid buffer with a preservative. The buffer solution elutes the drug from the pad and is used for laboratory testing.

Several devices use an absorbent pad or wad that is placed in a transport container that does not have a buffer or preservative solution. In this photo is an example of a chewable pad that is placed into the mouth of the donor until saturated and then placed into the transport tube.

At the laboratory, the tube is centrifuged to remove the oral fluid from the pad. The pad is removed from the transport tube to obtain the oral fluid at the bottom of the tube for drug testing.

Collection devices with absorbent pads, as those in the previous two slides, have advantages and disadvantages. Some of the advantages are that there is no direct contact with the biohazardous oral fluid, if you are using a paddle pad; easy manipulation of the oral fluid, if it is eluted into the buffer solution; and possible drug stability in the buffer solution. Some of the disadvantages include that the speed of collection can be slow for dry mouth, as found in some smokers, some drug users, or when the oral fluid is viscous. There is a lack of uniformity among devices in that some are time collected and others use a volume indicator and some have a buffer solution and others do not. Other disadvantages include possible inadequate volume for multiple drugs or for repeat analyses. In addition, there are a number of variables that affect collection device performance. Let us examine some of the issues that affect the performance of collection devices with absorbent pads. For the volume of oral fluid, an adequate amount is needed for multiple drug analyses or for re-analysis. Accuracy of the volume collected is important. As an example, if the lab analysis is based on the collection of one mL, but the actual amount of oral fluid is 1.5 mL, then the calculated drug concentration will be 50 percent higher. The standards set for pad devices should require limits on the variability of volume.

The pads must be evaluated for their capability to absorb the required analytes. Removal of absorbed analytes from the pad is a very important issue. Scientific studies reveal analyte recovery varies

among the devices. For example, in the study by Dennis Crouch and others, they found recoveries vary dramatically among six devices. Amphetamine percent recovery ranged from 16-97 percent, while cocaine performed better with a range of 61-95 percent. Langel and others performed recovery studies on ten devices. They found THC, a lipophilic compound, to have the lowest recovery of the tested analytes. The device with the poorest performance had less than 12.5 percent recovery; the device with the highest recovery had 85.4 percent.

Analyte stability is important with or without a buffer solution. Analytes should be stable for at least two weeks under refrigeration.

The next topic I would like to discuss is screening immunoassays, which are assays that use antibodies to detect the parent drugs and metabolites in oral fluid. Since the concentration of analytes is lower in oral fluid than in urine, screening tests will require greater analytical sensitivity. There are two types of immunoassays to detect drugs and metabolites in oral fluid. The first type is the heterogeneous enzyme immunoassay. The Enzyme-Linked Immunosorbent Assay or ELISA is commonly used for screening drugs in oral fluid. Their advantages include nanogram or picogram per mL sensitivity with small sample size of 25 microliters for each drug class. ELISA kits can also be very specific to analytes in a drug class. For example, there are some ELISA kits that are specific for amphetamine with essentially no cross-reactivity to methamphetamine. The disadvantages of the heterogeneous assay is that a wash or separation step of the bound and the free antigen is required before an absorbance reading of the labeled antigen. This process can increase labor costs, but automation procedures can reduce the labor cost when used for pipetting, washing, and the absorbance measurement.

The second type of immunoassay for analytes in oral fluid is the homogeneous assay. These assays do not require a separation step of the bound and the free antigen. Advantages of this type of assay are that these assays are designed for automated procedures that permit rapid throughput and are not labor intensive, which should lead to a lower cost per test. Disadvantages include the specificity, which can be an issue with cross-reactivity of over the counter medications, especially for the amphetamine class of drugs. Sensitivity is a challenge with homogenous reagents, in that the optical measurement is made in the presence of the biological matrix.

This slide shows the initial test analytes and the cutoff concentrations for oral fluid from the 2004 proposed revision to the Mandatory Guidelines, compared to the initial test analytes and cutoffs for urine from the October 2010 revised Mandatory Guidelines. Notice that the initial test analytes are the same. They include 6-AM and MDMA that were recently added to the urine panel, but the cutoffs for oral fluid are less than urine due to lower concentration of drugs and metabolites in oral fluid. Also included in this table are the manufacturers' immunoassay kit cutoff ranges for each oral fluid analyte. As you can see, the manufacturers' cutoffs are not standardized, with ranges that are as large as a ten-fold difference for THC and a kit specific to amphetamines. Some manufacturers offer separate immunoassay kits for amphetamine and methamphetamine.

For specimen validity testing, urine specimens can be adulterated or substituted by the donor due to the availability of adulterant agents and the nature of the unobserved collection process. Creatinine, specific gravity, pH, and oxidant testing are required on all urine specimens under the Mandatory Guidelines of April 2004 to detect an abnormal specimen.

The proposed Guidelines of 2004 for oral fluid recommended the determination of immunoglobulin G (IgG) concentration on every specimen. IgG concentrations should be greater than 0.1 micrograms per mL; concentrations less than 0.1 micrograms per mL IgG would indicate a substituted specimen.

Some scientists believe that the specimen validity testing is not needed for oral fluid specimen due to the observed collection process.

Moving on to confirmation testing. The gold standard for confirmation drug testing is an analytical method that combines chromatographic separation with mass spectrometric identification. The two most common types of analytical chromatography methods used to separate compounds are gas and liquid chromatography. Either can be interfaced with one or more mass selective detectors.

GC/MS was the only confirmation method permitted for urine drug testing under the Mandatory Guidelines from 1988 to October 2010. New technologies were permitted for urine drug confirmatory tests with the October 2010 revised Guidelines. Some examples of the new technologies include LC/MS, GC/MS/MS, and LC/MS/MS. Of the 38 currently certified laboratories in the NLCP, only two labs are using a new technology. Both labs are using LC/MS/MS for 6-acetylmorphine analysis, which has the lowest urine confirmation cutoff and is subject to interference by other opioids. Oral fluid testing will require these new technologies.

On my final slide is this table displaying the confirmatory test analytes and cutoff concentrations for oral fluids from the 2004 proposed revisions to the Mandatory Guidelines and compared to the analytes and cutoffs for urine from the October 2010 revised Mandatory Guidelines. Notice first that the analytes for oral fluid include two parent drugs not included in urine - THC and cocaine parents. It also includes the designer drugs MDMA, MDA, and MDEA, which were recently added to the urine panel. Also notice that the proposed confirmatory cutoffs for oral fluid are lower than those for urine.

The newer confirmation technologies will be useful for the analysis of THC and will certainly be needed for the carboxy metabolite of THC used in urine testing. If this metabolite is added to the oral fluid panel, these newer technologies will be needed since the concentration of this metabolite is usually in the picogram concentration in oral fluid.
Thank you.

Dr. Cook: I ask that the Board members please hold their questions until the end of this session. I will now like to welcome Dr. Courtney Harper of the Food and Drug Administration, who will talk about the FDA's position on oral fluid.

Methodologies (Collection Devices, Screening Immunoassays, Confirmatory Tests)

Dr. Harper: Thanks, Janine. I am Courtney Harper, and I work at the Food and Drug Administration. For those of you who are not that familiar with FDA, we do a lot of things, including the regulation of human and animal drugs; biologics, such as vaccines and tissues; food and food products; cosmetics; and also medical devices. I work in the group, the Center for Devices and Radiological Health, which regulates medical devices. In particular, our office regulates in vitro diagnostic devices. We perform one stop shopping, uniquely offering both pre-market and post-market regulation of laboratory tests. Our office has about 150 people. We review more than 1,000 submissions a year because we recently added radiology to our responsibilities. We also perform compliance actions. We oversee devices from the time that they are developed, to the time that they are evaluated for clearance or approval, and then, once they are on the market, we continue to monitor them and evaluate how they perform work. In our office, we regulate IVDs, in vitro diagnostics, which includes a broad range of devices used for diagnosis, screening, risk assessment, prevention, or surveillance. These devices are used in a broad range of settings, including central laboratories, over-the-counter use, and point-of-care tests. This is one of the reasons that FDA is an ex officio representative on this particular Board. My division regulates clinical chemistry and toxicology devices. This slide presents

the broad scope of the devices we regulate, including general chemistry tests, such as cardiac markers, diabetes tests, and toxicology tests, as well as therapeutic drug monitoring and drugs of abuse testing. Today I am here to talk about drugs of abuse test regulation. This table lists many of the drug tests that we often review at FDA.

These types of tests require FDA clearance prior to going to market. When a manufacturer creates a device, they send the device and its supporting data to FDA. We review that data to determine whether that device works as well as other similar type devices that are already on the market and FDA cleared. If the device is acceptable and there are no safety or effectiveness issues that are raised, then the FDA clears that test and the manufacturer can market that test.

In that premarket review setting, what do we look at? We examine three things, generally, for any in vitro diagnostic device. We evaluate analytical performance. Does that test measure what it says it measures and how well does it do that? We also assess to some degree its clinical performance. How reliably does that result actually correlate with the patient's condition or their status? For many tests out there, we only review analytical performance because, for instance, we don't need the manufacturer to conduct a clinical study to show us that monitoring blood glucose is a good thing to do if you are diabetic. Nor do we request that manufacturers prove that measuring cocaine is a good indicator that somebody has taken cocaine. Just because we have a clinical performance requirement does not mean that we actually review clinical data for some of these. Drugs of abuse tests, for instance, are more the exception than the rule. I will talk about that when we review some clinical data in a minute. The other requirement that we concentrate on and really believe in is labeling. Many of you here concentrate on and think about the testing process, such as the drug testing process, donor sample acquisition, transfer, etc. At FDA, we focus on the devices. How is the device designed? How well does that design achieve what it is intended to do? How well does the device perform for its intended use? Ultimately, we want the labeling to reflect that. If we determine that the device is safe and effective, then we want the labeling to say how this device works because no device is perfect. Some of the devices have a false positive rate of x and a false negative rate of y. Sometimes these rates are okay as long as the people using it can read that and are able to choose the device that works best for their needs. That is why we have a labeling regulation that requires the devices to have adequate instructions for use. This is used quite importantly in the drugs of abuse realm.

What do we ask for when we review a drug test for clearance approval? There are very general, commonly assessed parameters for any type of laboratory test. The same evaluations that you perform in your laboratories are the same evaluations from the manufacturers that we examine. We evaluate precision. Will you obtain the same test result from the same specimen over time? With point-of-care testing, it is especially important to evaluate the setting and types of people who will use it. Will you obtain the same result as other people testing the specimen? These questions evaluate the device design. How well is the device designed? How easy is it to use?

For performance around the cutoff, we request that the manufacturers evaluate specimens throughout the range to ensure that the device cutoff is not too broad so that the device will be reliable or predictable. We have them measure drug concentrations from zero to very high. We then focus our scrutiny on those specimens around the cutoff.

For quantitative tests, we evaluate recovery. Usually, these recoveries are not as good as those for quantitative laboratory tests. With accurate labeling, though, laboratories can better assess when they need to perform dilutions for confirmation to determine how much drug is in the specimen.

We require that the companies evaluate cross-reactivities. All drug tests have some degree of cross reactivity with metabolites or similar drug compounds. Again, this is reflected in the labeling so that the laboratories using the test will know what to expect from that particular test in terms of cross-reactivity. These tests do vary because of the antibodies used in any particular test vary in their degree of cross reactivity with the similar compounds.

We also ask manufacturers to evaluate interferences that are not metabolite or drug interferences. These interferences are matrix-dependent. We do not want an interference that creates a positive result out of nothing. That is, in a negative specimen, you do not want an interference that produces a false positive result. If the analyte concentration is near the cutoff, are there any interferences that either shift that result up or down enough to create a difference in the test interpretation in near cutoff specimens? If so, by how much?

In serum, for instance, hemoglobin and bilirubin are evaluated as interferences. We may request interference studies on common over-the-counter drugs, in addition to compounds of similar structures. We ask the manufacturer to evaluate compounds that are known to interfere with certain types of drug tests. In urine, we request that they have them also look at pH and specific gravity. In oral fluids, we also have them evaluate other interferences, such as blood, mouth wash, etc. We have seen these, especially blood, interfere with oral fluid tests based on colorimetric reactions.

Accuracy is the area where we see the most difficulty for manufacturers, partially because the studies we are requesting are not always that easy to do. We believe that they are necessary for an accurate evaluation of how the device works. We request that specimens be analyzed by the device and the confirmation reference method. We are not looking for confirmation as to whether somebody has taken drugs or not. We are assessing accuracy around that cutoff. This is an analytical evaluation of the test. What we have required, ideally, in a test like this, is many samples right around the cutoff, both above and below, to determine where that cutoff is analytically. Samples with concentrations further away cause the cutoff to perform much better. For instance, if a sample that is 80 percent below the cutoff, most likely it will be negative. If samples are 10 percent below the cutoff, some of those will be positive. Ideally, this is how the study should be performed, but it is very difficult to find samples in those ranges.

Because of the difficulty is collecting specimens around the cutoff, we also request precision studies. To prepare the precision study samples, the manufacturers were spiking to the target concentrations. We had noticed over the years that the performance data from real samples and the performance data in spiked samples are very different for drugs of abuse tests for many tests because of metabolites or other interferences. We do now require them to obtain natural, undiluted, unspiked samples to do this. Because of the difficulty in obtaining these specimens, we generally request that they obtain ten percent below the cutoffs and ten percent that are near negative. What we call near negative is fairly generous - from the cutoff to 50 percent below and from the cutoff to 50 percent above. We prefer at least that many samples in that range. We also recommend some zero specimens. We evaluate the positive and negative percent agreement with the reference method to assess accuracy.

With a point of care test, we also have them assess the accuracy and precision in the hands of the intended user, such as a less trained user or a nurse. Ideally, the user would perform the test in the course of their everyday operations, such as treating patients, where they normally would experience that kind of distraction. That is how you get the best idea of how well they are doing at the test. Generally, we have it across three sites there is no site-specific bias.

For over-the-counter studies, we request that they use lay users. We often let them use spiked sample and correlate those results to the accuracy study results done with the natural specimens. We want to evaluate how well a lay user can pick up the device labeling, read it, and perform the test without training like they would in an over-the-counter environment. We assess whether the lay user obtains the answers as professional users.

We clear many of these types of tests because the bar is quite generous. These devices are screening tests, and the positive results are meant to be confirmed because these devices often have more false positives and false negatives. The allowable number of false positives and false negatives is in the labeling. However, there is an allowable bar, and some devices do not achieve it.

Some performance issues that result in a device not being cleared include positive results when no drugs are present and there is no acceptable explanation and the cutoff set incorrectly. An incorrect cutoff issue is particularly common when you have an instrument reader for a visually read test strip. The manufacturer can increase the sensitivity on the reader to decrease the detection limit and to detect reactions that are not visible with the eye. This lowers the cutoff significantly. For instance, a visual strip screening test that was designed to have a cutoff of 1,000 for amphetamines would produce positive results on a reader at 200. You may not care about these issues in a drug screening program because you would confirm whether drugs were really taken. Our goal is to verify the labeling, for instance a test with a cutoff of 1000, by evaluating an analytical comparison. If we see consistent positives all the way down to 80 percent below the cutoff, we may request that the manufacturer readjust somehow by either lowering the cutoff to be a more accurate reflection of how the test actually performs or readjust the settings on the instrument reader to make it more accurately reflect the cutoff.

One problem is poor recovery of drug following pre-analytical steps. This is particularly true for oral fluid and also for hair testing. Another problem is too many false negative results at very high drug concentrations, such as at thousands and thousands of nanograms per mL above the cutoff. Often these are not explained. If there are too many of them, we will not label them.

The clinical part of this is when there is no cutoff that is well recognized, such as a SAMHSA-recognized cutoff or literature-recommended cutoff. In that case, we request that the sponsors provide us with some clinical information to illustrate to us what is going on with this cutoff. Usually, we ask the sponsor to go to drug clinics and interview drug users to collect information on how much drug they took, when they took it, and look evaluate that.

We cleared the first oral fluid drugs of abuse test about a decade ago. Since then, we have cleared many drugs for oral fluid testing. This slide provides a list of some of them. We have cleared many tests, as well. The vast majority are central laboratory-based tests, which are designed to be performed in the laboratory.

We have cleared only one or two point of care oral fluid tests. The oral fluid tests that were pictured in the slide by Dr. Walsh are generally not cleared by the FDA. Thus, their performance is not known.

The advantages of oral fluid testing are that collection is easily observable, the specimen is easy to collect, and these tests are often just as easy to run as a urine test. Listed here are some of the challenges. I will focus on these challenges in the next few slides, but I believe these challenges are ones that we can address. DTAB can easily make some decisions that might help us get to the point where we either know what to study or know what types of standards to set to solve some of these challenges. The challenges that we see when we evaluate a test are the ones you have already

heard today. One issue is that the collection method impacts test results. When a specimen is needed for confirmation, what is that specimen? How do you collect it? When do you collect it? How does variability in the specimen collection method impact the result? What cutoff to use do you use?

I will present a few examples from our experience. The first example is poor recovery using a collection device because with some of the drug is absorbed by the collection device pad, particularly THC. We have reviewed point of care collection devices for FDA clearance that had recoveries of less than ten percent. These devices were not cleared. One issue that needs to be addressed is what collection devices are appropriate to use for different drugs. We need to decide whether or not that type of recovery is acceptable. The drug absorption problem is an problem not only with pad collection devices but also with materials used to hold expectorates, such as certain types of plastic and glass. The tolerance for drug absorption will need to be tackled.

The self-contained collection for some point of care devices are designed to place the whole device in the mouth to collect the oral fluid. This is not inherently bad design in that it is easy to use and you know for certain that the specimen was collected is from the donor. The problem for us has been trying to figure out how and when the confirmation specimen is collected. This specimen cannot be removed from this collection device. Thus, the specimen tested by these devices is not the same specimen that is confirmed. Do you wait until you get a presumed positive to collect a confirmation specimen? If you are shipping the specimen, do you have them take a confirmation specimen? If you do, what do you put the specimen in? Especially for THC, many the plastic containers will not be appropriate.

These are issues that the manufacturers are currently not addressing, but we are asking those questions of them. It would be helpful if they had standards, suggestions, or guidelines to adhere to so that everybody was doing the same thing. Right now, manufacturers are either addressing these issues either in very different ways or not. The different ways that they are addressing these issues will lead to situations where the oral fluid results between labs will not be comparable.

The next example illustrates the issue with dilution imprecision. Many of the devices that are intended to easily collect oral fluid samples require placement of the collection devices into a dilution buffer. In most cases that may be the right thing to do because it may help preserve the specimen and stabilize the drug. This also provides more volume for easier method of testing and aliquoting. Remember, though, that these collection devices are designed very differently. We have seen collection devices that require either a one to one dilution, a one to two dilution, or a one to three dilution. These different dilutions will make the comparisons of results between oral fluid specimens collected at different dilutions impossible.

Another issue is what is the correct confirmation specimen. Do we want to determine the concentration of drug in a diluted sample? Or do we want to determine the concentration of drug in a neat specimen? Will be back calculate? That decision will matter because if the diluted sample is screened then it is easy to confirm because you have a diluted sample. But there will be different dilutions depending on what device is used. That makes it more difficult for a drug testing program if different dilutions are used. Recommendations for a set standard dilution or a standard method of determining dilutions and back calculating are better. These issues can be addressed. Currently, there is more variability than consistency out there. If the specimen is the neat sample, there is much variability because of the related recovery issues. If you have a device that is designed to do a 1:3, sometimes you get a 1:3.3, a 1:3.1, or a 1:2.5. These results are from the same study. We require the sponsors to perform a recovery study of the collection device, and that is the wide range of data that we see for volume recovery. This imprecision in the collection step will add to the imprecision around

that cutoff. When we consider what standards we want, we need to also address this dilution/neat sample problem and the issue of what is the nature of the sample that we are confirming. This also impacts the cutoff because if you apply a cutoff to a diluted sample of 40 and you apply a cutoff to a neat sample of 40, you are talking about two different things. 40 in the 3:1 dilution is really 120, whereas 40 in the neat is 40. Are we comparing apples to apples?

Based on our experience, the devices are designed in all of these different configurations. The labs are trying to discern which one to use. It would be helpful if we could figure out which ones we would like for them to use. All of them are doable, but people want guidance.

The last example is this issue of a cutoff. We had one device in which the sponsor submitted an application for a drug that we had not previously cleared in oral fluid. We were not able to find much information on the right cutoff to use nor could we find much information on oral fluid testing for this drug at all. As a result, we requested that they perform a clinical evaluation. What we found is that they were detecting legal use and not illegal use because of the retention time. They were detecting chronic use. If use was long enough ago and used every now and then and in vast quantities, they were not detecting it. We did not clear this device because the performance of this device was not appropriate for the intended purpose.

I have presented how we evaluate oral fluid tests and some of the issues that we have seen. I wanted to quickly demonstrate how you can find information about tests that we have cleared. Listed here are some websites. The Device Classification Database allows you to research the different types of tests that FDA regulates by product code. In the section called Device Advice, we provide information about how FDA regulates medical devices. Our 510(k) Releasable Database is where you can find the summary of the data that was used to clear any particular test since 2003. On the website are guidance documents, including our recommendations for studies and considerations and for over the counter labeling.

We really encourage people who are developing devices to speak with us if they have questions about what they should do or some issues about their particular device. The process they can use for that is Pre-IDE. You can contact me, and I advise on how to start that interaction. It is free, interactive, and flexible. It is a wonderful opportunity for us to learn about you and for you to learn about us.

Finally, I will walk you through how to find those summaries of cleared devices that I mentioned earlier, in case you want to review the data for any particular test. This is the website for the review summaries. At the website, you enter the device name, the applicant name, or the product code, which is the type of test. If you typed in cocaine in the device name, and a list of cocaine tests would appear. Once you select a device, you follow the link to the decision summary. If you click on that, a long document appears. On the first page is a summary of what the device is and near the end of this document is a summary of the data that was used to clear it.

In summary, rapid oral fluid tests for drugs of abuse are not yet widely available as FDA-cleared products, though they are available as laboratory tests. They provide a promising opportunity for drug testing. There are some challenges that we need to work on. We are really interested in helping companies overcome those challenges and also providing input to the DTAB. These devices are challenging to develop, but they should demonstrate that they work well and consistently enough for the purposes that we need to use them for. Thank you very much.

Dr. Cook: Thank you, Courtney. We will hold questions until the end. I welcome back Dr. Frank

Esposito, who will relate his experience with proficiency testing of drugs of abuse in oral fluid.

Proficiency Testing

Dr. Esposito: Hello again. The topic for this presentation is oral fluid proficiency testing. My objectives for this presentation are to report on our experience at RTI over the last four years with two PT programs. First, I will present the design and the results of the final year of the SAMHSA-sponsored Oral Fluid Pilot PT Program. Secondly, I will present the design and the results of the oral fluid program currently offered by the Center of Forensic Sciences at RTI.

First, I will detail the background of SAMHSA's Pilot Oral Fluid PT Program. In April 1997, a public meeting of SAMHSA's Drug Testing Advisory Board discussed drug testing in alternate matrices, including oral fluid. From 2000-2006, SAMHSA's National Laboratory Certification Program funded an oral fluid pilot proficiency testing program for up to 16 interested laboratories, some of which were non-NLCP urine labs. A total of 15 cycles of PT samples were sent to the participants.

An important finding over the course of the program was a large variation in reported results. In the final year of the pilot program, the laboratory variability and the stability of the oral fluid material was a primary focus. Let's examine the design and some of the results of the pilot program in its final year.

First, the focus of the study design was to evaluate the within and between laboratory variability over the course of the year. 14 laboratories participated, and they agreed to resolve any issues with testing accuracy and precision. Samples were prepared in a single production of neat samples in a synthetic oral fluid matrix. Samples were stored frozen at -20 degrees Centigrade between shipments. Three samples were sent to each laboratory at zero, three, six, and twelve months. The PT sample concentrations were 1.5 times the 2004 proposed initial test cutoffs. Each PT set contained two samples. In set one, one sample contained amphetamine and morphine at 75 and 60 ng/mL and the other sample contained methamphetamine and codeine at 75 and 60 ng/mL. Set two contained cocaine with MDA and benzoylecgonine with MDEA at the concentrations shown on the slide. The third set contained THC with PCP and 6-AM with MDMA at the concentrations listed. To facilitate the evaluation of immunoassay kits, cross-reacting compounds were separated in the spiked samples. Laboratories screened each sample once and quantitated each sample five separate times in five separate batches.

The results of this pilot PT program were compiled by RTI personnel and were presented at the Society of Forensic Toxicology meeting in 2007 by Peter Stout. This is an intersection plot of all of the laboratories reporting results. Each point represents the mean of the reported results for each laboratory over the entire one year study period.

Some analytes were more challenging for all of the labs. 6-AM, codeine, PCP, and THC had more consistent performance between laboratories than did amphetamine, MDA, MDMA, morphine, and methamphetamine. Most laboratories did not exhibit a consistent bias except for laboratory O, which did report, on average, lower results than the other laboratories for most of the drugs. This graph does not include cocaine and MDEA results, because three labs did not report these compounds.

The data are found on this graph, which is also an intersection plot for all of the laboratories reporting results for cocaine and MDEA. MDEA was more challenging than was cocaine.

This graph looks at the performance over the four testing events of the project, which is related to the stability of the PT material. Each point represents the drug mean concentration for all laboratories for

each cycle. If the PT material is not stable over time, you would expect to see a significant trend with each cycle. Each drug had consistent results, with no significant change across one year except for morphine. Morphine-reported results did not show a trend over time, but the results for cycle one were less consistent among the laboratories than in the latter cycles, two, three, and four. Cocaine and MDEA also had consistent results with no significant change over the one year period.

Now the variance of labs can be viewed by the mean percent coefficient of variation for all laboratories for each cycle. Here is the mean percent CV for each analyte for cycle one. Three months later, in cycle two, there was a sharp decrease in the variability, as laboratories were able to assess and improve upon their prior performance. At six months, or in cycle three, there was continued improvement for many of the analytes, however, there was increased variability for some analytes due to the individual laboratories. At the end of the year, in cycle four, the laboratories continued to demonstrate improvement for most drugs. However, for some analytes, results showed more variability, again due to the individual laboratories. This illustrates how much of a challenge it is for laboratories to maintain consistent performance over extended periods of time. As an example, it was observed that there was continued variability in benzoylecgonine performance. Some laboratories reported to us that their refrigerated calibrators were not stable for as long as they had anticipated.

For a perspective on the variability seen with oral fluid with this project, we can compare the oral fluid laboratory performance to the performance of certified laboratories in the NLCP urine PT program. The urine PT results show the variability in an established, mature program with over 20 years of performance testing experience. For most drugs, the oral fluid performance was comparable to the urine variability for at least one cycle during this study period with the exception of BE. Note that the THC in the oral fluid material is compared to THC acid in the urine material. One way of evaluating these data is to identify those compounds that are at or below 10 percent CV. Notably, BE was never below this line.

In summary, for the PT project conducted in 2006, the results demonstrated that synthetic oral fluid PT material is stable for all analytes for at least one year when stored frozen. There were significant decreases in the variability both within and between laboratories over the course of the study. Some analytes did have subsequent increases in variability in the latter cycles, illustrating the laboratory's challenge of maintaining its performance over time. While not discussed here, the PT material performed well using both GC/MS and LC/MS/MS procedures.

Now I will discuss the oral fluid PT program offered by our group at RTI. First, some background. As I mentioned earlier, SAMHSA funded an oral fluid pilot PT program through the NLCP for up to 16 laboratories from 2000-2006. In May 2007, the European Union Driving Under the Influence of Drugs, Alcohol, and Medicine, also known as DRUID, contracted with RTI to provide them with an oral fluid PT program for 12 laboratories in 11 European countries that would run until September 2009.

In December 2007, RTI provided an opportunity for the former SAMHSA pilot participants and other laboratories to continue in an independent oral fluid PT program. With the completion of the third year of the program in December 2010, there were 34 laboratories that participated in our PT program, 24 from the U.S., two from Canada, and eight from Europe.

The design of the oral fluid program is as follows. We prepare oral fluid samples in a synthetic oral fluid matrix to eliminate the biohazard risk and to provide for the stability of the analytes. The samples were formulated to contain three to five analytes from the 29 analyte panel of SAMHSA and non-SAMHSA analytes. Three mL of neat oral fluid are dispensed into 4 mL silanized amber vials with

Teflon-lined caps. Three cycles of five samples each are shipped each year. Samples for each survey year are prepared in a single production and stored frozen.

The oral fluid samples are shipped as neat oral fluid. The laboratories may analyze the samples as a neat sample or they can add it to an oral fluid collection tube with buffer, but the oral fluid collection pad must not be added.

In testing the samples, most laboratories first use an immunoassay method for their screening test. Five different commercial reagents were used by the laboratories. Quantitation and identification of the analytes are performed using a confirmatory mass spectrometry method. Analyte concentrations are reported back to us with values corrected for any collection tube buffer dilution. RTI sends a PT result form with group statistics back to each lab so the labs can compare their individual results to the group result and perform any necessary corrections.

This slide shows the 29 spiked analytes in our oral fluid PT program. In the first column are listed the amphetamines. The second column contains the opiate-related compounds. Third column has the benzodiazepines. The last column includes the commonly abused drugs, such as the Z or sleep aid drugs and the barbiturates.

This table displays the percentage of laboratories testing various classes of drugs by immunoassay. For the SAMHSA analytes, all laboratories tested for four or more analytes in this group. The next most frequently tested drug class was the benzodiazepines, followed by methadone, barbiturates, oxycodone, ethanol, and MDMA. No labs tested specifically for 6-acetylmorphine, but rather relied on the cross-reactivity of their opiate kit.

One way to assess the performance of an immunoassay reagent is with an analyte concentration that is just above the cutoff. We prepared samples with analyte levels that were between 1.4-1.7 times the SAMHSA proposed cutoff. In the first column are listed the SAMHSA analytes while the SAMHSA proposed cutoffs are given in the second column. Because not all of the immunoassay manufacturers offer the same cutoff, listed in the third column are the cutoff ranges for each analyte. In the fourth column is the PT sample concentration for each analyte, which is the overall average of the lab values for the sample. Remember, these analyte concentrations were targeted to be 1.4-1.7 times the SAMHSA proposed cutoff. The last column is the percentage of labs that reported the sample as positive by their immunoassay test and in parentheses, the number of labs reporting. All the labs reported PCP and morphine sample as positive by their immunoassay test, which was expected since the group mean value for each drug was above all of the vendor cutoffs. BZE had the next highest positive rate at 88 percent, but the group mean value was only ten percent above the highest vendor cutoff. Amphetamine, THC, and methamphetamine had lower screening positive rates, since the concentration of each sample was less than some of the vendor cutoffs. Amphetamine had the lowest rate, but also had the largest range in vendor cutoffs.

Thi slide examines the immunoassay performance for non-SAMHSA analytes. Since there were no standard cutoffs for these analytes, we looked at samples that had group mean concentrations at 1.1-1.4 times the highest manufacturer's cutoff. The analytes are listed in the first column with the immunoassay kit cutoffs in the second column. The third column is the analyte concentration, which is the group mean. The last column lists the percentage of laboratories, with the number of labs in parentheses, which reported the sample as positive by their immunoassay test. All of the laboratories testing for methadone, oxycodone, and ethanol reported these analytes as positive by the immunoassay test that was specific for these drugs. These results are expected because the group mean value for each analyte was above the manufacturers' cutoff. The other drugs listed here are the

benzodiazepines, with varying sensitivity. Diazepam had the highest sensitivity while clonazepam had the lowest.

The final graph shows the variability of confirmatory quantitative results between laboratories, which can be evaluated by the mean coefficient of variation or percent CV. For PCP, as indicated by the green bar, the oral fluid mean laboratory percent CV was 11.6 percent for samples analyzed in the RTI program over the last year. For variability comparison, included are the data for the 2006 last cycle of the SAMHSA pilot PT program, as indicated by the gray bar, and the urine NLCP PT program data over two years, as indicated by the blue bar. The variances for amphetamine and methamphetamine are similar for each PT program with larger oral fluid variances in the RTI program as compared to the SAMHSA program. The mean percent CV for BE in oral fluid is similar for the two programs and is about two times that of urine. For the opiates, codeine and morphine had similar variances in oral fluid and are lower than 6-AM. THC had the highest variance with a mean percent CV of 23.7 percent, as compared to the pilot PT program of about 14 percent. The urine percent CV represents the carboxy metabolite of THC in urine. Variance is less than 20 percent for all of the analytes in oral fluid except THC. For comparison, the mean percent CVs for all analytes in urine is less than 10 percent, which can be attributed to the additional experience that labs have in analyzing urine over oral fluid. In addition, the NLCP urine program is a remedial-based program, requiring laboratories to investigate quantitative errors, whereas the current RTI program is the self-improvement based program with no remedial actions or follow-ups.

In summary, for the RTI oral fluid program, SAMHSA analytes are most frequently tested by initial and confirmatory testing. There was a wide range of initial testing cutoffs offered by the manufacturers for these analytes. The variability of laboratory oral fluid results, as measured by the mean inter-laboratory percent CV for each analyte, was less than 20 percent, except for THC. The mean variances for analytes in the NLCP PT samples in urine were less than 10 percent. Qualitative and quantitative accuracy of laboratories could be improved, if a remedial-based program for errors was implemented in our program instead of the current self-assessment program. thank you.

Dr. Cook: Thank you, Frank. We are holding all questions. I would like to now introduce Barbara Rowland, who will provide some real world experience with oral fluid testing of drugs.

Best Practices Experience

Ms. Rowland: Hi. I am Barbara Rowland from Quest Diagnostics in Lenexa, Kansas. I am the Director of Laboratory Operations there. In the essence of time, I will talk as quickly as my southern accent will allow.

First, we test about 2,500-3,500 oral fluid specimens a day in the laboratory. Our all-time one-day record to date is 5,012 oral fluid specimens. For specimen collections, we use the Intercept Drug of Abuse Specimen Collection Device made by OraSure. The donor is asked to abstain from eating or drinking for ten minutes prior to collection. The collection pad is placed between the lower cheek and gums, and it rests there until it is moist, about three minutes. Previously, there was only a two minute collection required, which was then lengthened to three minutes. Currently, we have several clients that use a five minute collection process because they think it collects a little more oral fluid by doing so.

The donor places the pad in the specimen vial, which contains buffer. It is important to know your dilution factors and whether or not you are using neat oral fluid. This device collects approximately four hundred microliters of oral fluid which is placed in approximately 800 microliters of buffer to

create a dilution of one part oral fluid to two parts buffer or a times three dilution. The donor is required to snap off the applicator wand, place the cap on the specimen vial, and place the tamper evidence seal across it. The chain of custody is completed, and the device is packaged for shipping.

We have several client employers that want to collect two oral fluid specimens - A and B. This is not a split collection, as we know it today for urine, but a simultaneous collection.

When the specimen is received at the laboratory, it is processed similar to urine specimens. We verify the specimen identification numbers, check the integrity of the specimen seal, and assign it a laboratory accession number. Next, we snap the plastic tip on the end of the device and insert it into a specimen storage tube. We then centrifuge this tube for two to three minutes to elute the oral fluid out of the device into the storage tube. The original collection device remains inserted into this storage tube and serves as a cap or a seal for the oral fluid. This then also preserves the original tamper-evident seal and identification.

For initial testing, we use the Intercept micro-plate EIA (96 well ELISA). The parent tube is not loaded onto our Hamilton aliquoters, but the specimen storage tube that I described earlier is. We pipette a 350 microliter aliquot from that specimen storage tube and transfer that aliquot to the screening area for testing. This aliquot is then loaded onto the Hamilton for aliquoting into the 96 well plate. Sample volume for each drug is between 15 and 50 microliters, depending on the drug assay. Our plate batch size is 79 donors, 16 calibrators and controls -- eight in the front, eight in the back, and one blind quality control specimen, which adds up to 96 wells. Our controls are at 50 and 200 percent of the cutoff.

This slide gives our initial test cutoffs. Notice that amphetamine and methamphetamine are tested on separate plates. The cutoffs are amphetamine at 100, methamphetamine at 40, cocaine at 5, marijuana at 1, opiates at 10, and PCP at 1. IgG is our specimen validity test, which has a concentration of 0.5 micrograms per mL. Any specimen with a result less than that is retested using a second aliquot from the original tube. If the two results are less than that 0.5, then that specimen is reported as invalid.

The initial testing equipment includes the Hamilton aliquoter, plate reader, and a washer. We use a multidrop dispenser and Ufill reagent dispenser to add most of the other reagents to enhance our large throughput through the laboratory.

Our confirmation cutoffs are amphetamine at 40, methamphetamine at 40, benzoylecgonine at 2, the parent marijuana drug at 0.5, the opiates (codeine, morphine, hydrocodone, hydromorphone) at 10, 6-AM at 1, and PCP is 0.5 nanogram per mL. We also test for MDMA and MDA. The IgG is the specimen validity test. Confirmatory testing is performed on specimen from that original tube. Our extractors remove a quantitative aliquot from the original specimen storage tube for the extractions, either liquid/liquid or solid phase. We use about 150-300 microliters, depending on which drug is being confirm. Our quality controls are 50, 80, and 125 percent of the cutoff for confirmation.

Our confirmation methods are listed here: liquid/liquid extraction for amphetamines and THC and solid phase extraction for PCP, opiates, and cocaine. Our derivatives are HFBA for amphetamines, BSTFA for THC, methoxyamine and BSTFA combination for opiates, and HFIP for cocaine.

Our confirmation instrumentation includes GC/MS for amphetamines, cocaine, and D/L isomers. We use a Dean’s Switch for PCP, THC, and opiates for extra sensitivity.

The non-negative specimens must be kept in frozen storage (-20 degrees Celsius). Refrigerator and room temperature storage is not appropriate for the specimens. These specimens are placed in frozen storage for a year, just like the urine specimens.

As a laboratory director, I am glad to see there are more and more proficiency tests for us to enroll in and there are more and more accrediting agencies. We are accredited by CAP, through Forensic Drug Testing, and the State of New York. For the 2011 proficiency testing, we will participate in the NLCP, as well as the College of American Pathologists PT programs. The State of Pennsylvania has just begun its own oral fluid testing program, as well.

Laboratory challenges with the oral fluid specimen are specimen volume, whether or not to test for THC acid to eliminate contamination issues, split versus simultaneous collections, retests between the different labs with different limit of detections, and automation.

Dr. Cook: Thank you, Barbara. We are finally to our last talk for this session. I would like to welcome Dr. Barry Sample, who will provide us with real life data on oral fluid.

Data

Dr. Sample: Thank you, Janine, and for asking me to come and talk about some of our data. I am really excited to be presenting because this is the first really public forum where we have talked about these trends.

Urine and oral fluid are indicators of recent drug use, and hair testing in workplace drug testing is an indicator of repetitive use over about a 90 day period, assuming an inch and a half of hair was collected and tested.

The objective of this study was to compare the trends in positivity rates between urine and oral fluid. The source of this data is our routine workplace drug testing specimens. We have excluded all data associated with our rehab, clinical justice, and point of collection tests receiving confirmation testing only. Thus, we have true population data for both the urine and the oral fluid test. Between January 2005 and June 2010, there were 4.9 million oral fluid tests and 32.6 million non-regulated urine specimens included in the summary data.

By testing reason, 74 percent of the urine and 81 percent of the oral fluid specimens were pre-employment tests, 13 percent and 9 percent were random, respectively, and both urine and oral fluid were 6 percent for post-accident tests.

Because these are part of our routine urine and oral fluid screening, there may be several cutoffs represented here. For the illicit drugs indicated in bold, the primary cutoffs that were used are amphetamines at 1000, cocaine at 300, marijuana at 50, opiates at 2000, and PCP at 25. The assay manufacturers are also listed on this slide. For confirmatory methods, while the standard cutoffs predominated, occasionally other cutoffs were employed, particularly for urine testing. Methodology was a mix of solid phase and liquid/liquid extraction, either with or without derivatization, but all were by GC/MS analysis.

Oral fluid screening is by the OraSure Intercept system. These cutoffs are on-device, assuming a nominal times three dilution; these cutoffs are not related back to original oral fluid. In our experience, the actual volumes collected range anywhere from 0.1 up to 1 mL, which is essentially a saturated pad.

This slide of our confirmation methods is basically the same as the slide that Barbara showed, although we had other laboratories that were doing oral fluid testing during part of this study, and therefore, may have used some different technologies. There was a mix of liquid/liquid and solid phase extractions with either GC/MS or tandem mass spectrometry, as well.

The overall positivity rates between urine and oral fluid are really quite comparable. There is only a 4.7 percent difference between those two data sets. Considering that the urine data also include non-SAMHSA drugs in this overall positivity number, the two data sets probably are equal or maybe oral fluid might be slightly higher once you remove the barbiturates, benzodiazepines, and other prescription drug data. The overall positivity rates are holding relatively constant over the last five and a half years at a little over four percent.

For amphetamines, we do see some marked differences in oral fluid versus urine positives, with 62 percent more positives in urine than in oral fluid. One of the interesting points on this slide is that the urine increase is presumably related Adderall prescriptions, but the increase in amphetamine-only oral fluid positives is not to the same extent.

For methamphetamine, it is just the opposite. There are 43 percent more positives in oral fluid testing for methamphetamine than in urine. Interestingly, we do see the trends of declining methamphetamine positives in oral fluid that we have seen in urine over the last few years.

Again, it is evidence that urine and oral fluid really are quite comparable specimens. Differences in prevalence rates can be tightly tied to cutoffs. With the new DOT cutoffs, in the last quarter of 2010 we experienced a more than 30 percent increase in amphetamines positives. We predict that this difference that we are seeing today between oral fluid and urine could easily evaporate at the new 500/250 cutoff, as compared to the standard 1000/500 cutoff.

In the case of cocaine, not unlike what we just saw with methamphetamines, there is a 45 percent difference. Trending is the same in both urine and oral fluid, except for the 36 percent more positives that we have seen in the fourth quarter of 2010 under new DOT rules. As non-regulated employers move to these new cutoffs, this difference will evaporate.

Marijuana positives were nearly nine percent more in oral fluid than urine. What I find interesting are the differences that we have seen recently versus in the past. This is even more remarkable when you consider the shorter detection window that, at least theoretically, marijuana has in oral fluid as compared with urine. The difference in positivity rates over the course of the last two and a half years shows an interesting trend.

For opiates, we are seeing a 72 percent difference in positivity rates with oral fluid versus urine. This data can be a little misleading when you consider that the majority of our urine tests are only reporting codeine and morphine, whereas in our oral fluid testing, about half of these oral fluid tests included the reporting of hydrocodone and hydromorphone as part of the confirmatory process. If you were to compare expanded opiate urine testing to this expanded opiate oral fluid test, that delta between oral fluid and urine will be remarkably smaller. This slide shows the individual opiate drugs that are included on the confirmation panel that were reported positive as a percentage of all tests for opiates. For the individual analytes, codeine and morphine have relatively low positive prevalence rates. In the first half of 2010, morphine was positive for only five out of every 10,000 specimens, but hydrocodone was at 0.77 percent or 77 out of every 10,000 specimens. The trending is not unlike what we have seen in urine, although slightly lower positive prevalence rates occur in oral fluid than in urine.

Another interesting aspect is that we do not see any hydromorphone. It is not because we are not testing for hydromorphone; we are, but the positives are so minimal that it did not even rate a place on this slide. Considering the metabolic fate of hydrocodone, you wonder why we are not seeing any hydromorphone in the oral fluid specimen. Probably the most striking item is the number of 6-acetylmorphine positives in oral fluid. Four out of every 10,000 tests for opiates are positive for the 6-acetylmorphine heroin marker. Looking at it a different way, considering that two out of every 10,000 are positive for both morphine and 6-AM means that about 40 percent of the morphine positives also have 6-AM. Flipping that around, the 6-AM positivity rate is 0.04 percent and morphine and 6-AM is 0.02 percent. In the first half of 2010, 50 percent of the 6-AM positives also had morphine. We have a significant number of oral fluid specimens that are 6-acetylmorphine positive that have little or no morphine. Some of these specimens have detectable, but below cutoff, morphine. Some of them have no morphine above LOD, as well, in oral fluid. That is interesting in light of the data that is being generated on the urine side.

For PCP, positivity rates are very low and quite similar and seem to track together quite well.

In summary, both oral fluid and urine are providing insights into an individual's recent drug use and are exhibiting similar trends. While there are some differences in the positive prevalence rates, they are remarkably similar. Where there are differences to a large extent, some of that difference is driven by cutoff. To the extent that people use lower cutoffs for amphetamines and cocaine in the urine testing world, those differences will disappear. Again, there were significantly more positives for the heroin marker in oral fluid as compared with urine. Thank you.

Dr. Cook: Thank you, Barry. I want to thank everyone for attending our Drug Testing Advisory Board Meeting. I adjourn the meeting at this time.

(Whereupon, the meeting adjourned at 12:15 PM)