Substance Abuse and Mental Health Services Administration's (SAMHSA)
Center for Substance Abuse Prevention (CSAP)

Drug Testing Advisory Board

July 15, 2013 Meeting

SAMHSA Building
Seneca Conference Room
1 Choke Cherry Road
Rockville, MD

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Call to Order (9:05 a.m.)

Dr. Cook: Good morning, I am Janine Cook, the Designated Federal Official and Acting Chair of the Drug Testing Advisory Board (DTAB). I officially call this meeting to order.

Welcome, Introductions, and Opening Remarks

Dr. Cook: First I have a few announcements. For those of you attending onsite, a copy of the agenda is located at the back of the room. For those of you who are joining us remotely, a copy of the agenda was emailed to you. There are two changes to the posted agenda. Dave Mineta of the White House Office of National Drug Control Policy (ONDCP) is unable to welcome you today because of a change in his schedule. But we are pleased to introduce SAMHSA's new medical officer, Dr. Eleanor Katz.

DTAB has its own website located on the link shown on the slide. Posted on the DTAB website are the DTAB charter, the roster of Board members, and meeting information, including past, present, and future meetings. The minutes, proceedings, and presentations from the open session will be posted on the DTAB website sometime in the future.

For those of you that have any questions concerning material presented during the open sessions, we have two options for you to submit your questions to the Board. First, if you are attending onsite, three by five cards are located at the registration for you to record your questions. Please leave your questions with the staff that are manning the table. Secondly, if you are attending remotely, you can submit your questions by contacting the operator by pressing star one. Submitted questions will be considered by the Board during the closed session.

The public comment period is scheduled today to begin at 4:00 pm Eastern Daylight Time, although the exact time will be dependent on our progression through the agenda. Currently, there are nine attendees who are registered to make public comment. If anyone else wishes to give public comment and has not registered, you can register onsite at the registration table or notify the Verizon operator by pressing star one if you are connected electronically. The public comment period is restricted to the time allotted, and the time will be equally distributed among the commenters. Public comments will be included in the meeting minutes as well as in the transcript. If possible, please provide either a hard or electronic copy of your comments to be shared with

the transcriptionist to ensure that your comments are recorded accurately. The Board will not be responding to any public comments at this time, but we will take them under consideration in the closed session.

For our onsite guests, restrooms are located either down the hall to your right or past the guard station and left down that hallway. The registration table is located in the back of the room if you need any assistance. Located down the hall to the right is also a café which you are welcome to visit during our breaks. Also, restaurants are conveniently located nearby if you want to visit them during our lunch break. Please silence your electronic devices because these will interfere with both the audiovisual as well as the transcription equipment. For our off-site guests, you are participating in listen only mode. If you need to contact the operator please do so by pressing star one.

I want to welcome and introduce our DTAB members. Bobby Bonds, Larry Brown, Phyllis Chandler, Tony Constantino, Laurel Farrell, Greg Grinstead, Marilyn Huestis, Courtney Lias, Susie Mills, Jasbir Singh, and Steve Wong are with us onsite. Donna Smith and Jim Swart are joining us remotely today.

I also want to recognize our Division of Workplace Programs (DWP) staff: Ron Flegel, Jennifer Fan, Deborah Galvin, Giselle Hersh, Charlie LoDico, Coleen Sanderson, Hyden Shen, and Elaine White. I also want to recognize Phameca Morgan, our intern, as well as Bill Sowers, our contractor who manages our Drug-Free Workplace Helpline.

There are several other distinguished guests that I want to recognize: Fran Harding, Director of CSAP; Eleanor Katz, SAMHSA's new Medical Director; Barbara Spencer from ONDCP; Paul Harris from the Nuclear Regulatory Commission (NRC); Tom Martin from the Department of Defense (DoD). Patrice Kelly, Cindy, Ingrao, and Bohdan Baczara from the Department of Transportation (DOT): Denise Johnson-Lyles of the Food and Drug Administration (FDA); Tony Febbo of the Department of Justice (DOJ); and Ian Ruckers and Connie Foster from our Office of General Council (OGC).

We have scheduled the dates for the remaining FY13 DTAB meetings, which are September 10 and 11, 2013. The September meeting will be held by teleconference and in closed session.

I have an important announcement. For the first time ever, the scientific presentations during the open session are accredited by the American Association for Clinical Chemistry Advances in Clinical Chemistry Education in New Technology for six continuing education (CE) credits. If you want to receive these CE credits, please email me at janine.cook@samhsa.hhs.gov, and I will send you the information for you to receive CE credits.

Finally, I would like to introduce Ron Flegel, the Director of the DWP, who will be extending his warm welcome. Thank you.

Mr. Flegel: Thank you, Janine. Good morning. I would like to welcome you to our second annual meeting of SAMHSA's CSAP DTAB. First, I would like to thank everyone for attending today's open session. I look forward to this meeting, and I appreciate everyone being able to make today's meeting that will be focusing on the technical and scientific aspects of hair testing. I would also like to acknowledge ONDCP, our federal partners, OCG, DTAB members, DWP staff, and subject matter experts, and all the working groups that put countless hours into all the information that we have acquired.

Each day, DWP helps answer and guide decisions made for the Federal Drug-Free Workplace Programs within federal agencies. I believe it will be clear from today's presentations that we support SAMHSA's mission of reducing the effects of substance abuse in America through our workplace drug testing program.

As stated in the 2008 preamble, the Department of Health and Human Services (HHS) believes that the addition of alternate specimens to the Federal Workplace Drug Testing Programs would complement urine drug testing. Accordingly, HHS established a staggered timeline for issuing final guidelines that allows for further study and research of these alternate specimens.

Again, I would like to thank you for attending the DTAB meeting. Now I have the pleasure to introduce Fran Harding. Since May 2008, Fran has served as Director of CSAP. Also, as part of the Executive Leadership Exchange within SAMHSA, she served as Director of SAMHSA's Center for Mental Health Services for six months, thus expanding her behavioral health perspective. As a veteran of state government, Fran spent many years in the New York State Office of Alcoholism and Substance Abuse Services, where she was responsible for the developing policy and guidelines for alcohol, drug abuse, and gambling prevention, treatment, and recovery programs. She is also recognized as one of the nation's leading experts in the field of alcohol and drug policy. I am very happy to have her here with us today.

Ms. Harding: I too want to extend a welcome to those attending the second meeting of the SAMSHA's CSAP DTAB for 2013. I want to also recognize all of our federal partners from ONDCP, DOT, DoD, DOJ, NRC, FDA, National Institute of Drug Abuse (NIDA), OGC, and the Drug Program Coordinators representing the 119 federal agencies subject to drug-testing. I would also like to say welcome to our general public as well as CSAP and DWP staff, and anyone else I have missed.

Welcome. It is a serious welcome because it is a serious topic. Of all the areas that we work with here in SAMHSA, particularly under my guidance in CSAP, this meeting really delves into our direct services to our federal workers. We thank all of you for your assistance, your work, your recommendations, and going forward with the very important discussion today.

SAMHSA's DTAB is a scientific expert panel that recommends changes in the Federal Drug-Free Workplace Programs to SAMHSA's Administrator. The two recommendations proposed by the DTAB and approved by the Administrator are in the approval process for incorporation into the proposed revisions to our Mandatory Guidelines. Yes, government can be slow. These are significant enhancements to this regulatory program which is designed to deter illicit drug use in federal agencies. Again, I applaud the Board for this due diligence in adhering to the HHS decision published in the Federal Register (FR) on February 25, 2008, regarding alternative specimen matrices for the Federal Workplace Drug Testing Programs. The Department believes the addition of alternative specimens to the Federal Workplace Drug Testing Programs will complement its existing urine drug testing. However, because of scientific, legal, and public policy information for drug testing on alternative specimens is not complete as it is for current HHS urine drug testing program, the Department established a staggered timeline for evaluating these alternative specimens, thus allowing for necessary and further study and research.

As most of you know who have heard me in the past, SAMSHA's mission is supported by its eight strategic initiatives that comprise the current strategic plan. Under strategic initiative number one, prevention of substance abuse and mental illness, the Federal Workplace Drug Testing Programs are a part of SAMHSA's overall prevention portfolio. As we plan for 2015 and beyond with our next strategic plan, prevention will continue to be the first strategic initiative, and of course you will be a part of that. Currently, we have four prevention goals: emotional health, underage drinking, preventing suicide and suicidal attempts, and the misuse and abuse of prescription drugs; these also will be changed for 2015 and beyond. The recommendations proposed by DTAB directly support and align with this initiative. It is important for you to know that you are an integral part of SAMHSA's mission.

As defined by the Institute of Medicine, workplace drug testing is both a universal prevention program, defined as strategies that benefit the entire population, and a selective program which targets specific sub-groups. Approximately 400,000 federal employees and 12 million workers in federally-regulated industries are subject to drug testing in the workplace. Workplace drug testing is the largest universal prevention program we have here in SAMHSA, so we do take this job very seriously.

I have the pleasure and honor of introducing you to Dr. Eleanor Katz, the Chief Medical Officer for SAMSHA. She comes to SAMHSA from the University of California San Francisco where she was the Professor of Psychiatry and the State Medical Director for the California Department of Alcohol and Drug Programs. She is board-certified in general and addiction psychiatry and is a distinguished fellow of the American Academy of Addiction Psychiatry. Dr. Katz has been working in the field of addiction medicine for 22 years as a clinician, a

teacher, and a clinical researcher. Her specialty areas include pharmacotherapy for substance use disorders; clinical pharmacology of drugs and alcohol; drug interactions; cocaine, alcohol, and opiate medication development; and co-occurring HIV disease and addiction, just to name a few. She has participated in several SAMHSA-sponsored projects, so she was a part of the SAMHSA family before she even came to SAMHSA, including being the Medical Director of the Physicians' Clinical Support System for buprenorphine and Prescribers' Clinical Support System for opioid therapies. The National Training and Peer Support Programs for Physicians and Clinicians Training Patients with Clinical Need for Opiate Medications is also part of her resume. Perhaps more pertinent to this group, Dr. Katz served as the Medical Director and Chief Operating Officer for the Virginia Health Practitioners Intervention Program, which provided monitoring services to the Virginia Department of Health Professions for any licensed healthcare provider with an impairing illness in that state. Toxicology screening was a major component of that program and remains an interest of Dr. Katz. Please join me in welcoming Dr. Katz to her first, but not her last, DTAB meeting.

Dr. Katz: Thank you very much. I will not spend much time making additional remarks, except to say that I come to this meeting mainly as a clinician. For the first 15 years or so of my practice, I was not really aware of toxicology screening and its use in the workplace. When I became involved with the impaired health professionals in Virginia, this became the backbone of our monitoring programs, so I have always been very interested in this topic since that time. It is enormously important in terms of workplace safety, and for those who work with patients, it is enormously important for the safety of some of our most vulnerable citizens. I am really happy to be here, and grateful for the service that you provide to Americans in this area. I will listen today and learn. I thank you for this opportunity.

Status of DWP Initiatives

Dr. Cook: Thank you Dr. Katz. I do apologize to those of you who are joining us remotely for our technical difficulties in projecting the presentations. For the DTAB members, you should have copies of each of the presentations to follow along. We will now discuss the status of certain DWP initiatives. Charlie LoDico will provide an update on the Federal Custody and Control Form (CCF).

Federal Custody and Control Form

Mr. LoDico: I do have great news, so listen intently. I promise you that you will be satisfied with my presentation. One project that I am involved with is the CCF. As part of my presentation, I will discuss the CCF updates for 2013. Every three years, I must satisfy all of the Office of Management and Budget (OMB) directives, initiatives, and requests for CCF renewal. The current 2010 CCF expires September 1, 2013. The 2010 form itself has no content change, no changes to the fields, and no changes to the instructions. It should have been a very easy process of obtaining a three year extension from 2013 to 2016. One thing that was troubling for OMB was the manner in which the CCF is currently in use. As you know, the OMB's mission is to reduce cost and the paperwork burden for non-federal agencies and the private sector. In 2010, OMB placed a condition on SAMHSA for the next extension of the CCF: "In accordance with the GPEA, OMB set terms of clearance for the extension of the current Federal CCF as follows: Prior to the next approval of this package, the Agency (SAMHSA) shall provide a progress update on adoption of electronic forms in an effort to reduce burden. SAMHSA is encouraged to explore ways to convert the Federal Drug Testing Custody and Control Form (the Federal CCF) into an electronic form."

This mandate, to reduce the paperwork burden and to adhere to the OMB requirements for the 2013 CCF extension, became the mission of DWP. How did we achieve this? As we always do, we engaged our federal partners, our consultants, and industry that have interest in this service and our program. DWP convened a working group that included, among others, the federal partners. Through this working group, we established the standards for this electronic CCF (eCCF). The standards which we were concerned about included electronic signatures, non-repudiation agreements for digital signatures, third party software that would monitor federal CCF information, and the unique specimen identification number. We also worked with our federal DOJ Judge Advocate General (JAG), Frank King, who was very helpful in researching case law studies. He found only two cases with any discussion of an eCCF, and in both cases, the court ruled that its use was

appropriate.

Working group members included Ms. Kathy Patrick of American Solutions for Business; Dr. Jennifer Collins of MedTox; Dr. Barry Sample of Quest Diagnostics; Ms. Phyllis Chandler of LabCorp; Mr. Neil Fortner of the U.S. Air Force; Mark Snyder of DOT; Eric Quilter, a software manufacturer; Dr. Lappe, a software manufacturer; and Todd Shoulberg, a medical review officer (MRO). This working group was comprised of a cross-section of individuals who had an interest in the eCCF and believed in information sharing. The purpose of this group was to gather information. This working group first convened on January 27, 2012, with follow-up meetings in April, May, June, and finally August of that year to produce the finalized consensus agreement. DWP then took that information and made the best decision for the program.

The group discussion centered on the risks and benefits of eCCF. We also defined standardized definitions and terms and established the eCCF operational considerations, including for the program, the guidance, and the laboratory, as part of the effort to secure information in an electronic format. One consequence of this potential eCCF was that our guidance document would need updating; the MRO Manual would now include the eCCF or instructions for the MROs upon receipt of an eCCF. Listed here are the documents requiring updating, including the MRO Manual, the Collection Handbook, the Laboratory Checklist used to inspect laboratories, and the guidance for using the 2013 Federal CCF. The guidance for the 2013 CCF is posted on our website in a question and answer format on how to use the eCCF.

SAMHSA has an onsite liaison person who interacts with HHS and OMB. For the clearance process, we must provide our SAMHSA officer with a 60-day burden hours Federal Register Notice (FRN). This submission packet includes about eight attachments, such as the CCF, the Executive Order, the Checklist questions, and a supporting statement. The supporting statement and the FRN were published on April 30 2013 with a 60-day public comment period. In that FRN were burden hours calculated from the previous number of specimens analyzed at the drug testing laboratories, based on a one year average. These burden hours included approximations of the time to perform a collection, to do a review, to complete an application, etc. We asked the public to comment on those burden hours, including were they reasonable, unreasonable, or fictitious. At the end of this 60-day period, there were no public comments received on estimated burden hours, making it easier for us to proceed with the final step. I am happy to report that today, Monday, July 15, 2013, a FRN was published that allows the following: SAMHSA will authorize the use of electronic Federal CCF, the Public Burden Statement will be on a separate page of the electronic CCF, the Federal CCF instructions for the Privacy Act Statements will be a separate page or pages of an electronic CCF, bottle labels and seals will be printed separately and not as a part of Copy 1 of the Federal CCF, and Federal CCF Instructions to allow the use of electronic forms.

Now comes the hard part, the work, which is addressing the guidance. We have to provide specific details for using the eCCF. We are in the first phase, and within another two months, we will provide support to the laboratories, especially answering the many questions we expect to receive from them. Though this work is not done, we have the foundation. We will exert effort on our part to really commit to your needs. Thank you.

Dr. Cook: Congratulations, Charlie. Next, our Division Director, Ron Flegel, will discuss the status of the previously announced DTAB recommendations.

Previously Announced DTAB Recommendations

Mr. Flegel: Thank you Janine. I also want to thank Charlie for his work on the CCF.

I want to update the public on the previously announced recommendations from DTAB. The proposed revisions to the Mandatory Guidelines for Federal Workplace Drug Testing Programs are currently in the review process. These proposed revisions to the Mandatory Guidelines for both oral fluid and urine will serve to enhance this regulatory program, which is designed to deter illicit drug use in the federal agencies and regulated industries. ONDCP, by the inter-agency agreement that they provided to SAMHSA, has helped to support the development of these Guidelines and toxicology laboratory standards for detecting drugs and their

metabolites in oral fluid. While the focus was to develop the Federal Guidelines for Workplace Drug Testing, these Guidelines are also critical in developing standards that may be used in roadside testing devices needed for drugged-driving enforcement in the future.

DWP is currently revising the MRO Manual for the interpretation of workplace prescription drug results, specifically the synthetic opiates. DWP has also managed some special projects through the National Laboratory Certification Program (NLCP) to answer difficult questions regarding the interpretation of test results for both urine and oral fluid. Some of these special projects included two dosing studies, the first one a hydrocodone/oxycodone dosing study and the second a hydromorphone/oxymorphone dosing study. There are also other oral fluid special projects that are either completed or in progress. These include the effect of tooth whiteners on drug analytes, analyte stability in neat and buffered oral fluid, stability of oral fluid in collection device buffers, characteristics of immunoassays currently in use for oral fluid drug testing, and the confirmation methods for oral fluid drug testing. We are also evaluating the impact of these revisions on the certified laboratories.

We will continue to look to the future and hope to clarify and resolve the issues with other specimen types to allow their use in federal and federally-regulated workplace programs. I want to thank you again for attending. I will turn it now back over to Dr. Janine Cook.

DTAB's Process for Evaluating the Scientific Supportability of the Hair Specimen for Federal Workplace Drug Testing

Dr. Cook: Thank you, Ron.

In this presentation, I will discuss the process that the DTAB will use for evaluating the scientific supportability of the hair specimen for federal workplace drug testing. SAMHSA has seven advisory councils. DTAB is unique among those seven in that it is the only one that is a scientific council. This quote from the DTAB charter addresses SAMHSA's objective in having such a Board, which is that "SAMHSA seeks to improve the quality of services for forensic workplace drug testing, assess the science and technology used in drug analyses, improve the quality of related laboratory services and the systems for drug testing, generate standards for laboratory certification for federal workplace drug testing programs, and guide national policy in these areas by the establishment of the CSAP DTAB."

What are the duties of the DTAB? Per its charter, "the CSAP DTAB provides advice to the Administrator of SAMHSA based on an ongoing review of the direction, scope, balance, and emphasis of the agency's drug testing activities and the drug testing laboratory certification program. It shall recommend areas for emphasis or de-emphasis, new or changed directions, and mechanisms or approaches for implementing these recommendations. Periodically the CSAP DTAB shall review specific science areas and new drugs of abuse and the methods necessary to detect their presence." The key duty of the DTAB is to provide advice to the SAMHSA Administrator, and it does this in the form of recommendations. Their job is to monitor that science, and based on the science, to make recommendations. In July 2011, the Board proposed two recommendations, one for oral fluid and the other one for synthetic opioids.

Now I will provide a bit of regulatory history on hair testing. In April 13, 2004, proposed revisions to the Mandatory Guidelines were published. In that summary, HHS was proposing to establish scientific and technical guidelines for the testing of hair, sweat, and oral fluid specimens, in addition to urine specimens. When the final Guidelines were published in 2008, the summary only mentions urine. This slide depicts the statements found in the 2008 preamble. The key issues from the preamble regarding the use of these other alternate specimens mentioned in 2004 were that submitted public comments and additional comments provided by federal agencies during the subsequent internal review of the proposed changes to the Guidelines raised significant scientific, legal, and public policy concerns about the use of alternative specimens. What HHS expressed in that 2008 preamble is that the scientific, legal, and public policy information for drug testing of oral fluid, hair, and sweat patch specimens is not as complete as it is for the laboratory-based urine drug testing program. HHS found three issues with these alternative specimens that they expressed in the

preamble. First, the data from the pilot performance testing (PT) studies showed that all the participants in those programs have developed the capability to test for all the required drug classes with acceptable accuracy. Secondly, some of these drug classes are more difficult to detect than others and varies by specimen type. And thirdly, the specific drug classes vary by the type of specimen.

What is HHS's stated position as a result? HHS believes that the addition of alternative specimens to the Federal Workplace Drug Testing Programs would complement urine. These alternate specimens avoid the risks associated with urine specimens, including test suborning due to adulteration, substitution, and dilution. HHS stated that each of these alternate specimens poses different concerns. HHS recommended following a staggered timeline for issuing final guidance that would allow additional study and research for each of these alternate specimens. DTAB, as SAMHSA's scientific advisory board, will follow this staggered approach. HHS, through the DTAB, will continue to evaluate these alternate specimens and then issue further guidance in the FR.

The Board did complete its evaluation of oral fluid, and Ron provided an update on that. Today we begin our evaluation of the hair specimen. We will progress through the same steps that the Board followed in evaluating the oral fluid specimen to evaluate the hair specimen. For the first step, today we are tasking the Board to assess the state of the science of hair as an alternate specimen for the federal program. The second step is to review the historical perspective of hair as the drug-testing matrix, which will be presented by Dr. Mike Walsh after the break. Afterwards, we will discuss the current perspective of hair specimen drug testing, including the characteristics of the specimen itself; hair collection, preparation and stability; drug analytes; analyte stability; cutoffs for the initial and confirmatory methods; the methodologies for those; and proficiency testing. In addition, we have presentations on laboratory best practices for hair testing and actual hair drug testing data. Another step already in progress is an exhaustive hair specimen literature search, with several hundred articles already collected. For the next step, which began over two months ago, we identified questions associated with hair testing that will require more in-depth discussion by the Board. These questions were generated and shared previously with the Board for their review and input. The 50-60 final questions were then organized into the following categories: hair specimen; hair collection and specimen preparation; drug and/or metabolites and cutoff levels; specimen validity; initial and confirmatory testing; quality control and performance testing; and MRO review. During the closed session on Tuesday and Wednesday, the Board will discuss each of those questions in detail. For each question, the Board will determine if they have a consensus answer. The Board may decide that there is not enough information and request a more in-depth review of the literature for an answer. We might do as we had done with oral fluid, which was to publish a request for information (RFI). In the RFI, we list specific questions and ask the public to provide answers, especially those supported by data or the literature. The Board may also request additional research studies to be done. For instance, as Ron just told you, for oral fluid, many studies were conducted to answer those questions that required further research. Also, we may assign specifics questions to our appropriate federal officials to answer for which the Board does not have that expertise For instance, legal questions are directed to our JAG attorney and our OGC. Other examples are those questions forwarded to our NIDA and FDA representatives on the Board for their expert opinions. To emphasize, the Board is following the exact same process that it followed for oral fluid.

The next step, after the Board believes it has answers for all those questions that were raised, the Board will deliberate on the scientific supportability of the hair specimen. If you remember from oral fluid, that deliberation has to occur in an open session. When that will occur, I do not know, because it depends on how long it takes the Board to review all the science of hair testing and for the Board to be satisfied with that science. That timing may also depend upon whether additional research studies are needed because such studies take time, especially if they involve subjects. In addition, DWP must address all the scientific, legal, and public policy concerns, including those raised in 2004. Finally, the DTAB will either recommend or not recommend proposed revisions to the Mandatory Guidelines. These will be made, in writing, to the SAMHSA Administrator. If the Administrator approves those recommendations, DWP staff will then draft proposed revisions that will be reviewed by the Board and subsequently published in the Federal Registrar for public comment.

That concludes my presentation. We are a little bit ahead of schedule. It is break time; we will reconvene here in 15-20 minutes. There is a café down the hall that serves refreshments.

(Break)

Dr. Cook: Now we begin the Accent credit portion of the program. If anyone needs CE credits for their professional certification and that certification accepts Accent credits, you can earn up to six hours of CE beginning now until the public comment period. This slide shows my email address. If anyone would like ACCENT credits, please email me at the end of the day and I will send you the link to apply for Accent credits.

I want to introduce our first speaker of the scientific session, Dr. Michael Walsh, President of the Walsh Group. Dr. Walsh will be giving us a historical perspective of hair as a drug testing matrix.

A Historical Perspective of Hair as a Drug Testing Matrix

Dr. Walsh: Good morning everyone. My job this morning is to talk a little about the history of hair as a drug testing matrix and the history of the consideration of the federal government for including that matrix in the federal program. Some of the slides are a little busy because I am trying to do a fairly comprehensive job.

The use of hair for detecting various compounds goes back a very long time, almost 150 years, to 1858 when a gentleman named Hoppe published the first report of arsenic detection in hair. For the next 100 years or so, hair was used to detect toxic heavy metals and poisons, primarily in Europe but also in the United States (U.S.).

In 1977, Dr. Werner Baumgartner essentially invented what he called RIAH - which is an acronym for a radioimmunoassay for hair – when he was working at the Veterans Administration lab in Los Angeles. He converted solid hair into a liquid phrase and examined it like a urine specimen. He initially detected opiates, and this represents the first modern use of hair for the testing of drugs of abuse. Subsequently, Arnold, a German colleague, introduced RIAH in Germany, which generated some controversy. Hans Sachs, another German, wrote a very good article on the history of hair testing in Forensic Science International in 1997. He discussed the behind the scenes events of that time. He suggested that both Baumgartner in the U.S. and Arnold in Europe, in their zeal to encourage and engender enthusiasm for this new technology, made many claims that later did not hold up to scientific scrutiny. In some ways, this impeded the growth and acceptance of hair testing in the early days. The controversy that Arnold created centered on his testimony in many police cases in which he suggested that a positive opiate test in hair was conclusive evidence of heroin addiction. A few years later, other scientists realized Arnold was using the old Roche Abuscreen radioimmunoassay (RIA) which had high cross-reactivity to codeine. Sachs mentions a 1980 Klug article in which Klug began to confirm the RIA results with a chromatographic method, an important advance. Over the next few years, the use of gas chromatography (GC)/mass spectrometry (MS) with the mass selective detector improved detection sensitivity and specificity, which allowed many other drugs to be identified in hair. In 1995, the Society of Hair Testing (SOHT) was formed. They have annual meetings and offer a small PT program as well. Over these last 30 years, since Baumgartner invented the RIAH, there have been improved chromatographic mass spectrometric techniques, new methods of sample preparation and wash procedures, which have all improved the detection limits from the nanogram per milligram (ng/mg) range down to the picogram per milligram (pg/mg) range. That is a very brief history, but I will try to fill in some of the details in the rest of the talk.

In 1987, when I was running this program, we began considering the use of hair as a test matrix for the federal drug testing program. Numerous meetings were held between 1987 and 1990 with Dr. Baumgartner and others to determine the feasibility. I first became aware of this new RIAH technology via a letter from Nancy Reagan that was written to the Secretary demanding to know why NIDA was not giving Dr. Baumgartner more money to develop this wonderful new technology. In preparing for this talk by reviewing old files, I came across dozens of correspondence between Werner and I, and they were all very amicable and collegial.

In May 1990, NIDA, in collaboration with the National Institute of Justice (NIJ), sponsored a 2-day independent technical review. We contracted with the Society of Forensic Toxicologists (SOFT) to gather together experts in the hair testing field for this meeting. Participants developed a consensus report which at that time stated: “The

use of hair analysis for employee and pre-employment drug testing is premature and cannot be supported by the current information on hair analysis for drugs of abuse."

Later that year, the FDA issued a compliance policy guide for RIAH, stating that there was no FDA-regulated RIA product on the market that has been demonstrated to be effective in testing hair for the presence of drugs of abuse. At this time, we evaluated the technology and felt that it was still in its early stages and certainly would be premature at that time to include it in the federal program.

In 1992, SOFT held some additional meetings, revised their consensus document, but arrived at essentially the same conclusion. These unanswered questions indicated important deficiencies in the present knowledge of the analysis of hair. These deficiencies were delineated in this revised consensus report, which stated that because of these deficiencies, hair analysis alone would not constitute sufficient evidence of drug use for application in the workplace.

In 1993, the National Institutes of Standards and Technology (NIST) conducted a four round PT study at multiple laboratories. Results indicated a detection rate of 89 percent for correctly identifying drugs in those samples that actually contained drugs, but a five percent false positive rate was also found.

In 1994, DWP sponsored a second independent SOFT conference on Drug Testing in Hair in conjunction with their joint meeting with The International Association of Forensic Toxicologists, thus including international experts. Presentations indicated that too many analytical issues remained unresolved, which undermined confidence in the accuracy and reliability of hair test results for use in workplace programs.

Two two-day DTAB meetings convened in April and September 1997 focused on alternative specimens, with the hair matrix examined in many of the presentations.

In 1998, DWP formed an industry-led working group to advise on how hair could be incorporated into the federal program. Members included: Dr. Donald Kippenberger, Chair and either scientific or lab director at Psychemedics; Dr. Baumgartner; David Brill; John Irving; Ray Kelly; Tom Mieczowkski; Lance Presley; and Steve Van Nus. In addition to the DTAB meetings, this group held their own separate meetings, with many other experts participating in November 1998, January 1999, May 1999, and January 2001. The purpose of these meetings was to develop and provide recommendations to SAMHSA on how hair testing might be integrated into the federal program. The recommendations from this working group, to include hair as a test matrix in the federal program, were integrated into the proposed 2004 Guidelines, which Janine mentioned earlier.

From 2000 to 2007, RTI conducted a seven-year PT program, with seven hair testing labs participating in the 23 PT cycles. Initially, the results showed major inconsistencies across the laboratories, but over the seven year evaluation, there were significant improvements in most participants' labs on most of the analytes being evaluated.

In 2004, SAMHSA published the proposed Guidelines in the FR for review and comment. As Janine mentioned, in 2008 they published a final notice indicating that there were additional comments raised by federal agencies and public commenters on significant scientific, legal, and public policy concerns. Thus, urine remained the only authorized test matrix.

In 2009, RTI issued a final report under, I believe, a five year contract sponsored by DOJ on the ability of hair testing to distinguish between cocaine use versus contamination in hair. It was a fairly critical report and was followed a few months later by the Federal Bureau of Investigation (FBI) announcing they were suspending hair testing in non-criminal cases. A few months after that, Dr. Kippenberger, who had left Psychemedics and was now working the Army Medical Command, recommended to the Tri-Services working groups that all DoD labs cease hair testing.

My main point for these last five slides is to show that over the last 25 years federal agencies, such as NIDA,

SAMHSA, DOJ, NIJ, to name a few, have funded many grants and contracts to support research on developing the use of hair testing in both workplace and criminal justice programs.

This brings us to where we are now with integrating hair into the federal programs. The methods have improved significantly over the last 25 years. Lab performance has improved, the ability to detect small or minute amounts of drugs has improved with enhanced technology, and the criteria for what constitutes a positive test has also changed dramatically. A number of issues still remain unresolved. There are scientific questions and legal issues that have been mentioned. A number of these will be discussed in much greater detail in the presentations that follow. Of some concern is the mechanism, the basic anatomy and physiology of how drugs are incorporated into hair. Those mechanisms are still not completely understood in terms of the relative contribution of blood, sebum, and sweat. There remain issues with wash kinetics and the metabolic ratios. Still of concern is environmental versus external contamination. The issue of hair color bias is still questioned by some. The interpretation of test results, which occurs once a laboratory submits its report, is whether this implies use or exposure. Other issues of concern include the comparability of hair test results to other test matrices and the relatively low sensitivity to marijuana and the relatively high sensitivity to cocaine.

In terms of legal issues, which are more for DOJ than for DTAB, currently a number of state laws would prohibit or limit the use of hair as a specimen for workplace drug testing. The specimens that are authorized within the state for workplace testing are specified and hair is not among the list. Recently, there were two major decisions in a case against the Boston Police Department. Both lawsuits involved the same participants, 10 African American police officers who were fired for testing positive for cocaine in hair. This case has been under review for eight years since the papers were originally filed in 2005. The decision for the first case, which came down late 2012, was a Federal Civil Rights case alleging racial disparity, a constitutional civil rights issue. The judge, in his decision, ruled in favor of the Boston Police, stating that whatever bias may exist does not rise to a legal definition of disparate impact. In March 2013, essentially the same plaintiffs and the same defendant filed with the Massachusetts Civil Service Commission. The case focused more on the challenge to the reliability and the validity of the hair test to distinguish between use versus contamination. In this case, the Commission found in favor of the police officers, with most of them being reinstated. The crux of the decision was this quote: “The present state of hair testing for drugs of abuse, while potentially useful in clinical assessment settings, and in the context of child custody, criminal probation, and pre-employment hiring decisions, does not meet the standard of reliability necessary to be routinely used as the sole grounds to terminate a tenured public employee under just cause standards governing civil service employees under Massachusetts law”. I think this case is particularly important since the Guidelines are limited to federal employees. While DOT and NRC have extended the Guidelines’ reach, the original purpose of the Guidelines was testing civil service employees.

Another legal issue is the Americans with Disability Act (ADA). Current users of illegal drugs are exempt from the protections there, but people with a past history of legal drug use but no current use and have undergone rehabilitation are protected under ADA.

In terms of the comparability of test results between urine, oral fluid, and hair, we have some data. We did a MRO project with RTI and funded by SAMHSA. For this project, we had five years’ worth of data on urine, oral fluid, and hair from 2003 to 2007. The data are from all unregulated tests, predominantly pre-employment tests and included all test results. During that five year period, we collected data on 4.3 million urine specimens, about 650,000 oral fluid specimens, and about 47,000 hair specimens. These are not paired data, meaning that they are not from the same donors. Also, the specimens were tested at many different laboratories. About 50-60 SAMHSA-approved urine labs reported results. The majority of hair specimens were tested by Psychemedics and Quest. The majority of oral fluid specimens were tested by LabCorp and Quest.

Because these large numbers are a little bit disparate, I looked for general trends. This is a table shows specimen type grouped by the reason for the test. There are some differences here, though the majority of hair tests were for pre-employment, 95 percent versus 81 and 78 percent, respectively, for urine and oral fluid. The more interesting comparison is between these three different test matrices and lab positive test rates. Lab positivity rates were 4.09 percent for urine, 4.28 percent for oral fluid, and 10.53 percent for hair. The

frequency distribution of these positive tests is seen across the bottom. The oral fluid and the urine test results appear comparable while the hair test results look a little different. This difference may be related to window of detection. The matrix with the shortest window of detection is oral fluid followed by urine and lastly hair with its much longer window of detection. The fact that the labs are detecting up to two and a half times the number of positives for hair is not surprising. What is surprising is that the number of marijuana positives is fairly significantly less than cocaine. Across the other drugs, both oral fluid and hair yield higher rates of methamphetamine positives. In summary, urine and oral fluid specimens detect drug use over the last few days while hair detects over the last 90 days. Lab positive rates appear comparable between urine and oral fluid, but the rates for hair are higher. Frequency distributions are comparable for urine and oral fluid, and the frequency distribution of positives with the hair matrix are somewhat different.

In trying to come up with a way to summarize where we are right now, I decided it might be better to use someone else's words. The federal case dealing with the Boston Police did not deal with the science and technology of hair testing at all, but they focused in on this disparate impact of the results of the test. The Civil Service Commission decision from this past March said that "workplace hair testing for drugs as distinct from urine analysis has been and remains a work in progress, that there has been a longstanding debate within both scientific and law enforcement communities as to how accurately hair tests are able to differentiate between drugs found in hair due to ingestion as opposed to contamination by external or passive means. There are no uniform, nationally approved standards for hair testing. Protocols vary from lab to lab and have changed significantly over time. Depending on what protocol is applied, what laboratory does the testing, or what instrumentation is used, many appellants would test negative rather than positive."

Today is at the beginning of a new process for the DTAB to inform and discuss the current state of the art in hair drug test methods and to further explore the suitability of hair testing in federal drug testing programs.

Dr. Cook: Do any members of the Board have questions for Mike?

Dr. Brown: Mike, I am still trying to get my arms around the slide where you looked at urine, oral fluid, and hair. I am not sure I understand. I think you provided some disclaimers up front in terms of what the source of the specimens were. I am wondering whether those disclaimers might be as much of a factor as any other you articulated that might explain the differences, including that they were not from the same donors and the setting in which they were collected. I am just concerned that when we have data like this without the disclaimers clearly articulated in the slide that may give people some rise to speculation.

Dr. Walsh: What I was trying to look at a large general population of workplace drug tests derived from a number of employers in the country that were submitting specimens to the MRO that we were working with. These specimens were from over 9000 different companies from all over the U.S., with all states represented. The numbers of oral fluid and hair tests did increase each year during that 2003 to 2007 period. All I am suggesting with this comparison is, if you look at the big picture, there are many unknowns with these data. It could be that some of the clients of both the hair and the oral fluid tests had much higher rates of drug use, maybe from different industry bases. But we did analyze large numbers; the smallest number is 47,000 for hair, 650,000 for oral fluids, and 4.3 million for urines. I believe Dr. Sample will show some paired data where he has both the urine and hair from the same individuals. Probably 60 percent of the hair tests in this database were done by Psychemedics and 40 percent by Quest, and virtually nobody else. I have compared the Quest Drug Testing Index data on hair with the Quest data analyzed separately in my database, and they matchup very well.

I can recall a number of times that Dr. Baumgartner said that a hair test does not pick up single use. There must be a pattern of use, especially with marijuana, of probably three times a week every week to be able to detect marijuana (THC) in hair. That would explain the sensitivity to THC in hair, in the numbers I have seen, although I think the Quest data are showing about the same numbers for cocaine and marijuana.

From a big picture perspective, do not the SAMSHA national household surveys show that cocaine and marijuana use are about the same in the U.S.? I would be surprised. These are issues that the Board needs to

consider, whether hiring somebody based on a pre-employment drug test is comparable to firing somebody who is a tenured employee on the basis of a hair test? The issues become a little more complicated I think.

The Federal Government is in a unique position because of the constitutional provisions under which it operates. The Federal Government cannot do what a private sector employer can do with impunity. Maybe the best summary is it is complicated, it has always been complicated, especially now that we are down into the pictogram range – a nanogram is a part per billion, a pictogram is a part per trillion. I forget which president said we are starting to talk about real dollars now when you are starting to talk about that kind of money. But when you try to conceptualize one part per trillion, then you can easily understand the concerns about external passive contamination because it does not take much if you are testing down in the 50 picogram range, to produce a positive test.

Dr. Cook: Any other questions from the Board?

Mr. Bonds: Thank you. I, too, appreciate the way you chronicled the history. That really was very helpful. I want to ask your opinion on the ADA question. I think I understand the concept that someone could be rehabilitated but test positive because of the ability to detect a positive over an extended period of time. Is there any possibility to overcome that barrier?

Dr. Walsh: The technology does allow for segmental analysis of hair, which could give a better time range than sometime within the last 90 days. But others will have to talk to that issue. Frankly, I do not know how big a concern it is because I do not know if it has ever been litigated.

Dr. Cook: In this court decision, they discussed hair testing variability between labs. The NLCP is very good at standardizing lab performance.

Next, we will move into the current perspective of hair testing. Our first speaker is Dr. Peter Stout, who is a Senior Research Forensic Scientist at the Center for Forensic Services at RTI International. He will present the current perspective on hair specimen characteristics, collection, preparation, and stability.

Specimen Characteristics, Collection, Preparation, and Stability

Dr. Stout: My slice of the story is to talk about hair characteristics, collection of hair, preparation, and stability, which includes from growth of hair up to analysis. Other presenters will talk later about the analytical portion.

As a background perspective, I picked through and pulled out bits and pieces of research that I have done over about the last 15 years and that Jeri Ropero-Miller at RTI has done with me over the last 10 years. This work was funded by the NIJ. Other funding sources include HHS, the National Institutes of Health (NIH), and RTI internal funding. Shown here is a government disclaimer. Opinions expressed in this presentation are ours and are not necessarily reflective of any particular government agency, especially since many agencies were involved in this research over the years.

First, I will provide a framework about hair morphology, its characteristics, how it grows, how it is colored, what its chemistry is, and because of this morphology, what the incorporation pathways for drugs and other target analytes might be into hair. I will talk some about the collection of samples, from what body locations you would collect it from, and collection techniques. We will talk a bit about how you would prepare hair. Two big aspects of preparation are first, the decontamination of hair or how you mitigate potential external drug that is not reflective of somebody having consumed drug and secondly, matrix disruption or digestion. Hair is a solid and unlike oral fluid, urine, or sweat, it is not a solution. Somehow, the hair matrix must be taken apart so that the target drugs are removed from it and into solution. All of our analytical techniques are dependent on solution analysis. Finally, I will talk on the stability of drugs in hair.

To illustrate the morphology of hair, shown here is a schematic picture of a skin. As I later present thin histological sections, both human and mouse, keep in mind that skin is a three dimensional structure. Hair

follicles grow up from the dermis. At the bottom of this slide is the adipose layer of the sub-dermis, the middle orange layer is the dermis, and then the epidermis is at the top. To take a two dimensional picture of that, we slice through one of these hair follicles to view more of the structures involved in hair. Here is the epidermis and down there are sebaceous glands. These are the glands that secrete the oily waxy, mainly lipid, material that helps hydrate our skin to keep it supple. Typically in humans, these glands empty into the almost mature hair follicle up at the top near the epidermis. In humans, we also have apocrine sweat glands which typically are in association with hair follicles found in the armpits, groin, and perianal region but not really elsewhere on the body. Mostly in humans, exocrine sweat glands are usually separate from hair follicles, but sometimes they are conjoined with hair follicles. There is an outer root sheath, comprised of connective tissue, and an inner root sheath, which is fibrous material that is around the hair follicle. There is blood supply at the bottom in the dermal papilla. Notice the relative arrangement of hair shaft and the hair bulb there.

I could have made my title even shorter by saying, Hair: It is Not As Simple As You Think. It is a remarkably complex structure, from its growth and morphology to its behavior. Looking specifically at the bulb of the hair, which is the growing part of the hair, from the outside in, there is this connective tissue sheath, an external root sheath, and an internal root sheath. That internal root sheath interdigitates with the forming cuticle of the hair, and that is part of the structure that holds the hair into the dermis. When you pull out a hair, you will pull this entire follicle out because it interdigitates with that inner root sheath. With a cuticle cortex forming in the medulla, near the bottom around the papilla there is an entering blood supply, often in association with nerves. Here are melanocytes, which are the cells that extrude melanosomes, the little packets of melanins that are incorporated into the forming hair. Melanocytes are interdigitated throughout the keratinocytes, which are the cells generally forming the proteinaceous material of the hair. Melanocytes are integrally involved in hair growth, and as melanocytes change over the aging process, growth changes. As hair grays, it tends to be wirier, to grow faster, and to grow differently than hair that is still fully pigmented.

What I have tried to do on all of these sections is to provide a schematic so you can kind of keep in perspective what we are looking at. Think of this as a section of human tissue. This section just misses the dermal papilla, slicing through the side of the hair bulb. Notice the melanocytes with their fibrous appearance out through the keratinocytes. Notice the arrangement of these various layers of the forming hair from the outside adipose tissue of the dermis into the hair shaft.

Much of the information I will show is from mouse models because tracer studies are not possible in human subjects. This shows a mouse follicle with the same anagenic growth pattern and many of the same structures. What is different about mouse hair is that it has a honeycombed medula. Human hair has almost no medula. Animal hair, mouse hair in particular, has a lot of medulla, which is honeycombed, and cortex. Notice this ladder-like effect in the middle of that follicle section. The black structures in the middle are the melanosomes. Notice the outline of the papilla and these various layers in that anagenic hair follicle. I have been deliberately simplistic about hair histology; there are many additional complexities within the histology of hair follicles. Not only that, it changes over time.

The growth cycle of hair is typically broken out this way - anagen, catagen, telogen, and cycling back to anagen. Anagen is the actively growing phase, so it has those various structures associated with it. This is when melanogenesis and keratogenesis occur, with the hair being actively formed and growing out. The timeframe of anagenesis is variable between individuals, within individuals, and within sites on individuals. Through a process of apoptosis, which is programmed cell death, the follicle senesces and becomes what is known as a club hair. That stage of senescence is called catagen. Telogen is when this club hair resident in the skin is ultimately shed. The length of hair is controlled by how fast it cycles through. Somebody who is able to grow really long hair has a long anagenic cycle, but also a long telegenic cycle because that hair remains attached for a long time. This is different on different locations on the body. For the very short, very fine, villous hairs, typically characteristic of arms and body hair elsewhere, this cycle and the timing in that cycle are very different than on the head.

There is a misnomer in hair testing when it is referred to as hair follicle testing. Typically, the follicle is not tested. Most people object to having their hair pulled out by the roots. Hair follicles are used with DNA testing

because of the cellular material that is left behind. For other forensic applications, the follicle is used. There may be some toxicological applications where root material might be helpful. With hair testing, the mature hair above the scalp is cut and no tissue is taken from the root.

Shown here is a bright field micrograph of mature human hair from a brown haired individual. Notice the granular appearance to the color. These are the melanosomes that have been incorporated into the proteinaceous matrix of the hair. There is a bright line that runs parallel along the edge of the hair. The cuticle of the hair is largely amelanotic; it typically does not contain much melanin. That bright line is the light refracting through the cuticle around this round hair. With microscopic work, there is a plane of focus that provides a slice through the image, which is a three dimensional structure.

Hair is very amenable to scanning electron microscopy (SEM). Our SEM work was conducted using an environmental SEM. Part of the difficulty with SEM is oftentimes the sample has to be dehydrated because of the very high vacuum. Artifacts can occur because of the change in water content. Environmental SEM operates at near ambient pressures, so far less sample preparation is done to these hairs. We have hair that is more characteristic of what that hair would look like in the actual environment, rather than in the conditions of very high vacuum with palladium coating and other treatments. We have performed SEM of hair to detect morphological differences in different slices through populations of different hair types. This is picture of a fairly typical human hair. Our interns pretty much went blind one summer tying knots in hair and doing SEM work. This hair has a knot tied into it, introducing a stress-strained region around the hair. This allows us to examine what happens to this cuticle when it is tightly bent. It stretches on one side and crunches up on the other. This scalar appearance of the cuticle is very characteristic. The cuticle scales or cells are layered like tiles on a roof, encircling the entire hair. They are a very integral part of what the hair is and how it behaves in the environment.

This human hair is freeze-fractured. This hair was dipped it in liquid nitrogen, snapped, mounted, and imaged. The snap provides a nice way of examining the inside of the hair and how it behaves with a fracture running through it. That jagged part in the middle is the cortex. Notice the very fibrous nature of the cortex. Cells are laid down to make the hair. In laying down these cells, they are taken apart and de-nucleated, creating these long keratinous fiber formations that run through the hair. Similar to the rind of an orange, there is the cuticle that runs around the whole strand. Notice the sharp delineation between where the cuticle is and where the cortex is. Examining the cuticle closer, you notice that layered effect of the cuticle. Notice the very sharply delineated four to six cuticular scale layers with very clean breaks between them. There is a very clean break between the cuticle and the cortex. Again, I am trying to show the complexity of this structure. Hair is not just a protein fiber. There is the complexity of the chemistry of the fiber, how it is arranged both at the micro and macro scale, and the different foldings. This is another freeze fracture image of a hair, showing the fibrous nature of the cortex and the very sharp delineation of the cuticle. Looking closer, notice the four or five layers of the cuticle. There is a difference in the electron density of each of those individual scales. They are a little lighter on one side, a little darker on the other.

This image is from Clarence Robbins's book, Chemical and Physical Behavior of Human Hair, of which he has published several editions. There is an enormous volume of work on the characterization of hair that has been done for the cosmetic and textile fiber industries. Wool is a hair. There is a wealth of information about how hair behaves, how it accepts dyes, and how it behaves physically for various cosmetic treatments. Some of this work is adjacent to toxicology and small molecule chemistry. This is a transmission electron micrograph of an ultra-thin cross section of hair. Typically, the hair is osmium tetroxide stained, and then an electron beam is shot through it rather than bounced off of it. At very high magnification, notice there is a complexity even within the cuticle scales of these cuticular layers. There are several hypothesized models for the actual structure of the cuticle layers. Remember that each cuticle cell has been dehydrated, pressed down, de-nucleated, and modified in its formation, but its cellular components remain. There are parts of cell membranes, phospholipids, parts of cell mechanisms, other proteins, other lipids, and other cellular components still present and making up these layers. Then there is the complexity of what happens between these cells.

The chemistry of hair is not easy either. Hair is exposed to the environment. By being exposed to the

environment, it is very sensitive to the relative humidity of the environment. Hair may be up to about 30 percent by weight water, depending upon the relative humidity. Water is an integral part of how hair behaves, what it does, and what its chemistry is. Though water is a huge part of hair's chemistry, but it certainly is not everything. About 65 to 95 percent of hair is protein, and the rest consists of lipids, pigments, and other trace elements. These other components may be small mass-wise, but they may be large chemistry-wise. The other compartments of where things can reside within hair add to hair's complexity.

The protein component is composed mainly of a class of proteins called keratins, which can be either classified as hard or soft. These are typically high in sulfur content. There are many different differentiations of keratins. Though I say keratins like it is a simple term, it is not. In Clarence Robbins's book, there are about three chapters just on the chemistry of hair. There are complexities in the folding of keratins, especially in their secondary and tertiary protein structures.

Then there is the pigment. Non-cosmetic pigment is largely color that is naturally present in hair due to a class of compounds called melanins. Melanins are another highly complex group of hydroxyindole polymers that are formed in melanosomes from reactions with tyrosine. In biological systems, they exist as melanin-protein complexes, creatively called melanoproteins. There is a whole other class of proteins that are associated with melanins. Melanins are a collection of different polymeric states – monomers, dimers, trimers, and tetramers. These melanins are very electron dense, with their associated electro- and free radical chemistries that play in their formation. The eumelanins contain less sulfur and are blacker in color. Pheomelanins have more sulfur content and are more red/yellow in color. If you are blonde or red-headed, you have more pheomelanins. If you are black haired, you have more eumelanins. The quantity of these melanins determines hair color. Giuseppe Prota made it his life's work to research melanins and melanogenesis. He published an enormous number of articles and several books about melanins and melanogenesis.

How do drugs enter into the hair? For the physical migration of drugs, there are those pathways that are reflective of endogenous drug, meaning the drug traveled through the bloodstream to enter into the hair, and exogenous drug, in which the drug did not travel through the bloodstream to enter into the hair. The two cross paths add complexity of this. Endogenously, there are mechanisms of diffusion; active transportation out of the bloodstream; being incorporated into melanin; entrapped within the hair; physical entrapment in the melanosomes; energy-dependent transportation of these compounds into the hair follicle; and ionic, hydrogen, or covalent binding of drugs into hair, particularly the melanins, because melanin undergoes free radical chemistry that primes it to create adducts. This may occur when the cells actively growing. It also may occur higher up on the shaft while the shaft is still forming, with diffusion in from the sides of the follicle. There are a variety of different ways.

To give you a visual of this, here is a mouse model that was administered rhodamine, a fluorescent dye. Rhodamine was administered via injection, and skin and hair samples were collected from these animals at time periods ranging from minutes after dosage to weeks afterwards. This slide depicts a slice that is within about 20 minutes of the dosage. The schematic shows the hair bulb off to the right, and the mature hair off to the left. The bright structures are the external root sheath and the forming hair shaft. This is imaged via a process called incident fluorescence. A light of known wavelength is shown onto the sample. If the dye is present, it absorbs that energy and emits at another wavelength that we monitor. If the dye is not present, you get black. If the dye is present in this case, you see pink. The dye is in the hair shaft and the external root sheath within minutes of dosage. Looking a little closer, you can start to see this really quite fibrous appearance of what is stained in that forming hair shaft. This appears visibly like the dye is associating with forming keratinic fibrils in the forming hair shaft. Looking at the mature hair, there is a lovely demonstration of bands of compound within a hair. I will give you three guesses how many doses this mouse had. He had three doses. This represents growth over about three weeks, with individual doses over about three weeks. This nice banding pattern shows that after the dye enters into the blood stream, it shows up as a band in the hair. Also, we did not have contamination of these hairs with urine because otherwise you would expect a more diffuse appearance. Mice were housed individually in elevated cages to prevent that. Looking a little closer at those bands, notice a very diffuse staining of the mature hair structures. This is what I mean by that honeycombed appearance of mouse hair. That checker boarding is the honeycomb inside of the hair. This non-pigmented

BALB/c mouse does not have any melanocytes, yielding very diffuse staining all the way through the protein component.

To explore other ways of how substances enter the hair, we conducted radio tracer studies over the years using a process called auto radiography. We administered tritiated flunitrazepam, nicotine, cocaine, analogs of methamphetamine, a variety of other serum constituents, and a variety of different compounds. The dose is administered, a skin section taken and prepared, that skin section is placed on a slide, and a very thin photographic emulsion is laid over that section. The slide is stored in the refrigerator to develop over the course of weeks. As the radio tracer decays, it emits a high energy particle into that emulsion and a silver particle is formed. The film is developed and stained to distinguish the tissue structures. The film is then placed in a scope where light is shown onto the slide. Wherever the silver grain is seen is wherever radioactive emissions occurred, and it is where our tracer went. In this section is an anagenic hair follicle from of a C57 pigmented mouse. In the left-hand frame is the bright field microscopy. The dark areas are where the melanosomes and melanocytes are. The hair papilla is at the bottom. On the right, we see where the tracer went. It is very strongly associated with the melanin. By comparison, here is an albino BALB/c mouse subjected to the same protocols, same drugs, and same time points. There is still radiation there. There are still positive traces, but they are very diffusely spread out and not really associated with any particular cellular structure as in C57. There are many different studies with many different systems showing many different drug associations with melanin. This is a consistent finding, whether in brain or other different pigmented tissues.

What if you have a drug that incorporates into the sebum or sweat? It has exited out of the skin and onto the mature hair from the outside. This would probably have a very different appearance for this deposition pathway, but is it really possible? Mice do not have sweat glands, but they do have sebaceous glands. Shown here is a cross section through a hair follicle sebaceous gland in a mouse. The hair had been growing through the bottom part of that oval structure there, with the sebaceous glandular material sitting at the top. When illuminated, it is positive with much of the tracer being deposited in the sebum. This strongly indicates that, at least with flunitrazepam in this system, there is a potential mechanism for drug to move from the bloodstream, through the sebum, onto the hair. If mice did sweat, the odds would be good that we would detect drugs in sweat. Therefore, there is a mechanism by which drug can move through sweat to the hair. These are mechanisms that would involve going through the bloodstream to get to the hair.

What if the drug does not necessarily need to go through a bloodstream to get to the hair? Drug could deposit directly on the hair, or drug that is deposited on the skin or near the hair is washed onto the hair by sweat. This exogenous deposition could be smoke, liquid, solid, and many things. Hair is exposed to the environment, and the environment will dictate how these things move around.

In this is a mouse model, the same rhodamine tracer was administered endogenously. A pattern of deposition was seen. When that same tracer is applied externally to mature hair, very different pattern of deposition is seen. It is running along the edges of those cuticular scales now. One thing I do not show here is when we try to remove it, the same pattern remains. With various different decontamination mechanisms, the same pattern is seen. With cut hair and the dye applied in solution, there was bulk movement of the solution into this hollow hair, with staining of the insides of these hollow areas. It is a very different pattern microscopically than when the drug was incorporated into hair. Towards the right, notice those cuticular scales above where the bulk flow of the staining solution made it into that hair.

What does it look like in humans? Here are a variety of different stained human hairs. The one on the right is stained with rhodamine, producing a very similar pattern along the edges of the cuticle scales. We have done this study with a variety of different dyes with different chemical features to mimic potential target compounds. The dye is methylene blue. The bright field at the bottom is a blonde hair with very nice staining along those cuticle scales. The upper one is incidence fluorescence with methylene blue, which has a fluorescence band which is great with dark hair which light cannot pass through. It will fluoresce on the surface to indicate where it is staining. Notice the very similar pattern along those cuticle scales.

If that was not complicated enough, over the length of a hair strand, nothing stays the same. At the root end,

shown on the left side, are little areas of potential damage, where the cuticle has been scraped off by cosmetic treatment and wear, and tear. Moving along the strand, out towards the tip, more and more changes to the cuticle are seen. It appears like the cuticle has been knocked off, but now the staining is very different.

There is a different association of that dye, depending on the condition of the hair. Complicating factors include color, chemistry, morphology, condition, age, and environment. There are many different factors to control when trying to understand what is going where. Hair has a very complex growth process. This is hormonally, age, and genetically controlled. It is a very complex maturation process. There are multiple inter-related deposition pathways. Deposition pathways may be both reflective of drug that went through the blood stream as well as drug that landed on the skin and was washed into the hair by sweat. Melanin appears to be a major route of association. Endogenous and exogenous depositions have differences, but I am not sure what that might mean. Though we can see some visual differences, but chemically I am not sure I can identify the differences in those depositions. Morphology changes with time, age, wear and tear, environment, and all these other things.

For sample collection, most people recommend collecting hair from the back of the head in the occipital region. That is the place on humans where hair is most consistently in anagen, is growing, and is reflective of the growth of hair from the skin. Hair from the arms, legs, beard, etc. are not of the same consistency in the number of hairs in anagen, how fast they are growing, and so forth. Head hair grows about one centimeter per month with about 80 percent of hairs in growth. The arm is probably the next most socially acceptable place to collect hair, but its growth is much less predictable than on the head. Other body areas are options, but now the complexities of apocrine sweat, urine contamination, saliva contamination creep in. What does that mean? There is a lot of discussion around orienting the strand. A section of hair close to the scalp is desired. The first three centimeters from the scalp are a more optimal hair sample to avoid some of the damage present on the tips of hair. Looking around at the hairdos on most of us men, it is tough to keep short hair oriented. This can be a real challenge for collectors. There are also discussions on plucking and snipping. Most collection suggestions are more towards snipping than plucking.

For sample preparation, decontamination has been a hot topic. Across the literature, a there is little or no consensus on decontamination, and there is very little information on relative efficacy. There are almost as many decontamination methods as there are papers published on hair. They range from no treatment at all to very complex, probably very effective multi-step, multiple different solutions, and mathematical overlay of complex procedures. I cannot tell you which of these is more effective than others because there is very little information about that. There are very few studies that have been constructed to effectively deal with that.

The same applies to mechanical preparation. To snip or not to snip? Many papers discuss pulverizing the hair, subjecting it to ball mills and other various grinding mills, making powders of the hair, just finely snipping it up, and just cutting it so it is big enough to be shoved into the glassware and that is it. Again, for relative efficacy, I do not know.

The chemical digestion of hair involves the disruption of the hair matrix in order to extract substances out. There is very little consensus around this, with very little information as to relative efficacy and relative efficacy for any variety of compounds. In the literature, digestion ranges from no treatments whatsoever, to some kind of extractive step, to combinations of detergents and pH treatments, to solvents to break up the hair, and everything in between.

It is difficult to conduct studies to do this. Hair testing in unknown samples dictates techniques like MS, which is the workaday mechanism for testing hair. It is commonly available, it can distinguish chemical structures, it is great for identifying that, but it can only analyze what is extracted. The drug has to in a solution and away from the solid to be able to analyze it. MS is more amenable to human studies. Radio tracers, at least for efficacy studies of decontamination and extraction, are very difficult to do without knowing the complete mass balance of where the drug is in the hair and how much of the drug is in the hair, in such a way that is independent of my ability to remove it from the matrix. Radio tracers are particularly useful, especially high energy radio tracers, because melanin quenches and tritiated tracers can be difficult. Higher energy emitters put human subjects off

limits. It is useful in determining where that tracer is and how much of that tracer is in hair in total.

Tritiated flunitrazepam and nicotine were administered to albino and pigmented mice. On the left are the non-pigmented results for both flunitrazepam and nicotine. A sodium sulfide solution was used to disrupt the hair matrix and to produce a visibly complete disruption of the hair. About 80 to 95 percent of the tracer-associated radiation was in solution. In the albino mice, this would be great because it would be very amenable to MS analysis. For the pigmented mice, as with the albino mice, the hair was digested, centrifuged, and the supernatant removed. A small pellet remained. The supernatant was subjected to scintillation counting. The pigmented mice had at least 20 times the radiation present than in the non-pigmented mice for both nicotine and flunitrazepam. Vastly more drug was present in the pigmented mice. Only 74 percent of the radiation associated with nicotine was in the solution, and only 35 percent of the radiation associated with flunitrazepam was in solution. Now, a lot if not the majority of the tracer not available to a MS technique because it cannot be removed from the hair to analyze it. This will confound understanding where substances are going if you are not using radio tracer studies. But tracer studies are difficult; they are long, arduous studies; and they almost uniformly must be animal studies. The higher the energy emitter the better, which makes them more dangerous.

In summary, for sample prep there are many different procedures described for decontamination, physical prep, and chemical digestion. There is not a consensus on what is the best method. There is some consensus that some kind of digestion is needed to mitigate the potential external non-representative drug. But what that is, I am not sure.

For stability, a group examined the cocaine contents of 1000 year-old coca-chewing Peruvian mummies. Cocaine and benzoylecgonine (BZE) were found. But those mummies do not get out much. They are not getting haircuts, they are not dying or bleaching their hair, they are not going to the beach, they are not standing around in traffic with lots of ozone, they do not go for swims, and they do not do those things that affect their hair and the contents of their hair. That may be significant, or it may not be; it is very difficult to say.

The PT materials that we have made were created by external application of drug. We do see some changes in them over time, but they are largely very stable over the course of years. Without environmental impacts, hair may be stable. But not too many people are electing to live life like a Peruvian coca-chewing mummy. You will do things to your hair, and you will be exposed to things that will change what is in hair. So what does that mean?

Another study we did involved the in vitro external application of drug onto hair locks. We collected hair locks from African American (AA), blonde Caucasian (BC), and dark Caucasian (DC) individuals. There were about 40 individuals in each of these groups. Hair locks were obtained from each individual. Eight mg of cocaine was applied on the hands and then rubbed onto these locks. The locks were then subjected to hygienic treatments each weekday for a period of 70 days. We had interns that had the pleasure of washing hair locks and taking samples from those once a week. Over the time course, we saw a change. Shown here is the ratio of BZE to cocaine over time. What was proposed in 2004 was a ratio of 0.05 of BZE to cocaine.

Without doing anything else to the hair other than washing, storing it in the lab, letting it dry out, treating it nice, over time the blondes crept up over that 0.05 mark at about 21 days. We have seen this in other iterations of this same protocol we have done. Others have shown the growth of BZE. Yuji Nakahara in 1996 performed a lovely study in which he administered labeled BZE and labeled cocaine in a mouse model and demonstrated that what deposited in the hair was cocaine, even if you increase the BZE area under the curve. You would expect BZE to enter into the hair, but actually it was cocaine that was deposited in the hair. The BZE that was detectable in the hair came from that cocaine, not the BZE in the blood supply. Something is going on here. What that may be is that BZE is retained more than cocaine. It may be that BZE is more differentially extracted than cocaine. It may be that BZE is generated in situ in the hair from cocaine. I do not know, but it is changing over time. Environmental exposure causes changes that may impact what you are able to interpret.

Hair is complex. It is highly variable between individuals, within individuals, over the lifespan of individuals, and

between gender groups. It is also highly variable, but unpredictably so, between ethnic groups or any human subpopulations. It is not terribly characteristic either. Morphologically, I could not distinguish an African American hair from a Caucasian hair microscopically.

There exist multiple, complex, interrelated pathways for drug interaction with the matrix. There are many different ways that drugs can enter into hair, onto hair, and transfer from on hair into hair. These pathways overlap, and there are many different ways for this to occur. There is relatively little consensus about preparative methodologies for analysis. With that, I thank you very much, and I appreciate your time.

Dr. Cook: To remain on schedule, I ask the Board to hold their questions for Peter. Next I would like to introduce Dr. Christine Moore, who is Vice President of Toxicology Research and Development for Immunalysis Corporation. She will discuss hair/drug analytes, analyte stability, and analyte cutoffs.

Drug Analytes, Analyte Stability, and Analyte Cutoffs

Dr. Moore: Thank you for the invitation. I have been tasked with discussing cutoff concentrations for the analytes and stability of drugs in hair. I will relate these to the proposed Guidelines from 2004. I also had a couple of ideas for further consideration if the Board would like to discuss these afterwards.

Shown here are illicit drug data from emergency rooms (ER). What is very interesting is that the reported drugs are the five classes that are generally known as the NIDA five. They are all still relevant in our society. Phencyclidine (PCP), which many say is never found, is seen by the ER staff. This panel continues to be a good one for the workplace program.

These are the proposed cutoffs for initial screening. Immunoassay is essentially what is used, but there are other methods of screening which are being considered. Starting with immunoassay, in the left-hand column is the DTAB 2004 proposal. Next are other professional societies and their recommended cutoffs. The European Workplace Drug Testing Society in 2010 and SOHT this last year recommended an immunoassay screening level. The other societies provided cutoffs for three other drugs: methadone, buprenorphine, and benzodiazepine; these are not relevant to this discussion. The screening cutoffs are depicted here graphically. For opiates and cocaine, all groups are in agreement. There is a discrepancy for amphetamines and a huge discrepancy for cannabinoids. The reason for that is that the DTAB cutoff concentration is for tetrahydrocannabinolic acid (THCA), which is the tetrahydrocannabinol (THC) metabolite in hair.

Basic drugs incorporate well into hair. The basic parent compound, for example, cocaine, incorporates to a greater extent than their less basic metabolites, i.e., BZE. Hair immunoassay should target cocaine. For urine immunoassays, if cocaine is targeted then the degree of conversion of cocaine to BZE in the method must be measured. The former holds true for heroin users. Predominantly, morphine-3-glucoronide and a little bit of free morphine are found. In the hair, 6-acetylmorphine (6-AM) and morphine are detected. The immunoassay should target 6-AM or the degree of conversion to morphine must be measured.

THC, interestingly in urine, it predominantly present as the carboxy-THC form, which is the target. In hair, THC is from the parent drug. This is in contrast to what happens for oral fluid. Parent THC is also present in high concentrations in oral fluid. Hair immunoassays are targeting carboxy-THC, which is present at lower levels. This is also true with oral fluids, but the approach to those from an analytic point of view is a little bit different. Acidic drugs, like the carboxy metabolite, do not incorporate well into hair. Since the THC itself is found in higher concentrations, why not perform an immunoassay targeted to that? These assays are certainly reliable within the program, even though there are some that perform the carboxy method. The confirmatory procedure identifies the carboxy. For confirmation, on the left we have the DTAB recommendation as it appeared in the FR. The PCP cutoff was 300, opiates were 200, cocaine was 500, metabolites were 50, amphetamines were 300, and THCA was 0.05 pg/mg.

Examining this graphically, some of the other societies have included ethylene methyl ester (EME), which is not part of the 2004 proposed cutoffs. The amphetamines are all higher than those proposed by the U.S.

government. The THCA is multiplied by 10 because otherwise it would not be visible.

Are the cutoffs appropriate or relevant in any way? For PCP, only North America suggests its inclusion. I had doubts about that, but after looking at the ER information, it certainly is relevant in society. In 1997, Dr. Nakahara studied eight PCP users. The positive concentration range was from 330 to 14,000 pg/mg. The suggested cutoff appeared appropriate.

For cocaine, there is good agreement between the professional societies. They all mandate the detection of the metabolite in the confirmation. However, BZE and EME are not indicative of digestion because cocaine degrades to those. Cocaethylene and norcocaine initially were thought to be indicative of use, but those apparently are present in street cocaine. None of these seemed to be markers of ingestion. There was some discussion of the meta-hydroxy and para-hydroxy BZE metabolites a few years ago, but I have not seen anything for a while on that particular topic.

The best way to set the cutoff is to determine concentrations reported in users. The Lachenmeier group in 2006 studied about 100 cocaine users. The ranges are shown here for cocaine. Even though the low end is at or just below the cutoff, the average of 2610 pg/mg for cocaine, over 1000 pg/mg for BZE, and 270 pg/mg for cocaethylene is well above the recommended cutoff. Actual users are at really high concentrations. That was also the conclusion from Vignali et al, an Italian group who looked at self-reported cocaine use. We all know the drawbacks to self-report. If there was no drawback to admitting cocaine use, then there might be helpful information. People who use cocaine daily had cocaine in their hair at concentrations well above the 500 pg/mg cutoff. If you imagine a line 10 times less than that at 50 pg/mg for the BZE, then those concentrations are also much higher than what has been proposed. Even folks who use once or twice a month are easily detectable at this cutoff. There is no problem with this particular cutoff; it will identify cocaine users easily, even those who are infrequent users. That would seem reasonable.

For amphetamines, there is discrepancy between professional societies. Polettini's group last year studied some volunteer amphetamine users. They were dosed at low and high concentrations. The maximum concentration that they found was between 600 and 3500 pg/mg for methamphetamine with the corresponding amphetamine at 100 to 300 pg/mg for the low dose. The high dose is shown here. The ratio between the two was around 0.15, with a median of 0.13. The 2004 proposal was a cutoff of 300 pg/mg with at least 50 pg/mg pg amphetamine. That ratio is 0.16. That would also seem reasonable and in good agreement with what we are seeing in real users. That cutoff would seem to be reasonable for methamphetamine and amphetamine. While you might not detect infrequent users like you will with the cocaine because those were maximum concentrations, they do seem reasonable.

There was a case reported of a young 16 year old girl who was who was given ecstasy at a party without her knowledge. A hair specimen was collected a couple of months later. Her hair was positive, but at a variable level. Had it been collected closer to the incident, presumably the concentration would have been higher.

There is some area for discussion on amphetamines, specifically, a lower threshold. The 2004 proposal cutoff is higher than those of other societies. Maybe the Board would like to discuss lowering it a little bit. The methamphetamine with amphetamine as a metabolite appears to be appropriate. Methylenedioxymethamphetamine (MDMA), methylenedioxyamphetamine (MDA), and methylenedioxyethylamphetamine (MDEA) are included also. The Board should consider the ratio requirement for MDMA to MDA similar to amphetamine to methamphetamine.

For THC, there is limited disagreement between the societies. The parent THC is in higher concentrations than the metabolite. Screening for parent THC is allowable in other organizations. There is a definite consensus that the carboxy metabolite minimizes, but does not eliminate, the claim of passive exposure, which of course is very important for marijuana, which is the most widely smoked drug in our culture. Perhaps the Board could consider allowing a THC screen and include the carboxy for confirmation.

For opiates, there is good agreement between all societies that the detection of 6-AM identified heroin use,

which is a huge advantage over urine. We are all familiar with the issues of finding a good concentration for 6-AM in urine because the morphine levels are so overwhelming, even after heroin use. So hair is definitely better for this particular drug. In 2005, the Musshoff group reported opiate concentrations in hair from subjects in a controlled heroin maintenance program. They studied people that they knew were taking the heroin. They enrolled 46 known heroin users with all different hair colors. 100 percent were positive for 6-AM, but only 89 percent were positive for morphine. The 100 percent for 6-AM was interesting. The dotted line is the suggested cutoff concentration of the 200 pg/mg. Both the mean and the median of these users were much higher and easily detectable. That particular suggested cutoff would definitely appear to be appropriate.

What about the other analytes? Additional drugs are being considered for the urine and oral fluid matrices. These should be a potential addition to the hair program also, particularly hydrocodone, hydromorphone, oxycodone, and oxymorphone. I was encouraged to hear Janine say that they had research done with hydrocodone and hydromorphone for the other matrices. Presumably, they are collecting hair also because there is not very much data available on these drugs. I was quite surprised when I reviewed the literature to find that there is not really much information on these drugs, certainly when compared to cocaine and marijuana.

Researching my own database back to 2006, I had data from self-reported heroin users. We take the self-reported part with a pinch of salt as always. These folks said that they used heroin up to 14 times per week, which is about twice a day. The morphine is well correlated with how many times they reported heroin use. The correlation is 0.86, and that is quite astonishing. The 6-AM correlation of 0.58 was between the number of times the people reported use and the concentration detected in the hair. What was interesting was hydromorphone and hydrocodone were both detected in the hair of quite a few of the heroin users. Besides 6-AM and morphine, hydrocodone and hydromorphone were also found.

Some of the users also self-reported codeine use on a daily basis. The individual denoted by the circle reported taking both hydrocodone and codeine separately on a daily basis. The other four only took codeine. Hydrocodone was detected in all of those samples. Though it would seem that I had gotten them backwards, those that are light codeine users, shown on the left side, should have the lower concentrations in the hair but had higher concentrations. Well I double checked and it is right. We are of course still dealing with self-report. What was most interesting was there was not any morphine found, only the codeine and hydrocodone.

Of those nine self-reported heroin users, eight provided hair samples that were positive for morphine and 6-AM, while the ninth subject reported use only once a month. Six of those samples also had codeine, five had hydrocodone, and two had hydromorphone. For the five subjects that said they took codeine on a daily basis, morphine was not detected in any of them. The summary of that little project was the presence of morphine suggests heroin or morphine intake. Tylenol 3 would not work with hair. Morphine is found if Tylenol 3 is taken. There seems to be an interesting potentially linear relationship there between the reported frequency of heroin use and both the morphine and the 6-AM concentrations.

For oxycodone and oxymorphone, again amazingly, there was not very much literature. For oxycodone in hair, I found eight citations in PubMed. None of those studies dealt with drug users but discussed analytical methods. For oxymorphone in hair, I retrieved four citations and two of them were related to surgery on cats and dogs. It is interesting; vets must use oxymorphone, and I do not know why they tested the hair. One reference was a general screen, and one had a real patient, but it was more focused on tramadol and fentanyl than oxymorphone.

I tapped into my databank and reviewed the work we did in 2007 with Wayne County. I also did this with Montgomery County back then. We obtained hair from post-mortem cases. In this particular study, we had 14 total cases, but I will only show three. The hair samples were obtained from deceased individuals. There was narcotic paraphernalia present at the scene, the decreased had history of previous drug overdose, or there was witnessed drug use. Obviously, the deceased were targeted for drugs. Specifically, acute, not chronic, drug intoxication was detected. That was an interesting take on the cases. The lab did regular postmortem work, and they sent us hair samples to analyze.

In the first case, the heart blood concentrations are given in the middle column. Highlighted are oxycodone and hydrocodone because these are the drugs in which none much is found in the hair literature. Notice how high the concentrations are for cocaine, oxycodone, and hydrocodone in the hair. Other post-mortem findings in the urine include EME, cocaine, Diltiazem, Levamisole, oxycodone, and clonidine, which is a blood pressure medication. The ruling of the coroner was cocaine use and the manner or death was accident. Interesting is how much more information the hair gave than the standard post-mortem analysis.

Found in the liver of case 3 were morphine, hydrocodone, and lots of oxycodone. Oxycodone was also found in the hair at over 10,000 pg/mg. Cause of death was determined as oxycodone intoxication, and the manner of death was an accident. In this case, acute drug intoxication was suspected.

In case 12, oxycodone, hydrocodone, and 6-AM were not detected in the spleen in the post-mortem sample, but morphine and codeine were. Hair yields added information in such cases. The cause of death was the opiate use, and the manner of death was ruled as an accident in Wayne County, Michigan.

To summarize, there were fourteen cases. This table shows that the hair provides additional and great information. There is a lot more drug in there than is found in a traditional sample. Of course, it depends on which traditional sample you have. Overall, especially the cocaine, oxycodone, and some of the opiates, hair is a great matrix that can provide all kinds of good information.

To summarize the additional drugs or additional analytes, the post-mortem case demonstrated that the oxycodone, oxymorphone, hydrocodone, and hydromorphone were detected in hair quite often when the more traditional specimens were negative. This implies that it is relatively straightforward to include those in any workplace drug testing program. These drugs are being considered for the other matrices, there is no problem with those being detected in hair. There is just not that much literature, so more research is needed.

The final area that I was asked to discuss was drug stability in hair. I was really surprised that there is not much on this. Several papers say hair could be stored for years, but there are not much data. Some of the conditions for storage include in the dark, cool, no plastic bags, and no refrigeration. There is a surprising lack of data to support anything. One lab has PT samples that they have had stored, but those were external applications which may be different than real specimens. The United Kingdom (U.K.) is requiring or mandating authentic hair for PT, so that will be interesting to see how that will work.

I thought I would add a couple of items that the Board may want to discuss in addition to those three areas that I reviewed this morning. Because hair is a solid matrix, it is not as easy to determine the consistency of drug recovery as it is from a liquid. The extraction efficiency from authentic hair, because it is a solid matrix, should be part of any method validation. Other areas include the extent of drug conversion during its extraction, stability in storage and transportation, and the addition of other analytes.

Shown here are data we collected about four or five years ago on extraction efficiency from an aqueous extract. There are many ways to extract the drugs from the hair, including organic, acid, base, and enzyme. Percent recovery was calculated after one hour, two hours, and then three hours after incubation. Extracts were tested to the point where no further drug was removed from the hair. This should also be measured with method validation.

I mentioned earlier about the degree of conversion. What the immunoassays screens for it does not matter because it is converted to whatever the target is for the screen. For the confirmation, it does matter. It is critical for interpretations based on drug to metabolite ratios and what that means. You must know how much drug is converted, such as 6-AM to morphine for example, within your methods.

There are different considerations for method validation for hair than for the other matrices. Stability must be demonstrated under various conditions, including during transportation. Janine said that research generates more questions, thus opening up other areas from which more results are obtained. If a stored sample needs

reanalysis, that implies segmental analysis. Is it a different segment, was it collected at the same time, or was it a split specimen? Even though I really like Peter's graph of three doses to white mice, segmentation is really controversial. Re-analysis, after some time, will raise quite a few issues that the Board will have to discuss. Good luck to the Board. Finally, I reviewed the addition of potential other analytes to the program to harmonize with the other matrices.

To summarize, the proposed cutoffs do seem appropriate for PCP, opiates, and cocaine. I think amphetamines and cannabinoids may require further discussion. The analyte list should be in harmony with other matrices. Drug stability in hair needs far more research.

Hair is certainly a good alternative to urine and oral fluid. It gives very different information, and often better information. Its use should depend on what the reason for testing. Method validation may have some different requirements from what we are used to with the urine and oral fluid matrices, and those should be considered by the Board. I thank you very much.

Dr. Cook: Do any members of the Board have questions for Christine?

Dr. Huestis: In your slide that is entitled Cocaine Users, could you provide us with the median data in addition to the presented mean data? I think that will be very helpful.

Dr. Moore: I will get it to you.

Dr. Huestis: I have one more question, also on amphetamines. I believe you referenced our paper, but I cannot remember all the details. One thing I want to caution about is the ratio of methamphetamine to amphetamine in those specimens that had both methamphetamine and amphetamine in a matrix like oral fluid. For oral fluid, we did not feel that the amphetamine should be included because both early detection and late detection would be missed. I am assuming that in hair, where the incorporation into specimens takes so much longer, that was not an issue. I want review that data and make sure that the majority of the specimens had both analytes. Probably they did, but we should think about that one.

In the self-report of cocaine use, that is really interesting. Could you say a little more? Are the two to four times per week group and the one to two times per month all self-report?

Dr. Moore: Yes.

Dr. Huestis: Okay, very interesting data.

Dr. Moore: Do you want me to send you any of those papers?

Dr. Huestis: We can retrieve them from the information you have provided if we need to. Thank you. I have another question. Christine, why would you think that morphine would have a higher correlation than 6-AM? Are you worried about degradation of the 6-AM? Why do you think it would be higher with morphine?

Dr. Moore: It seems to be a time-related issue. It takes longer for the morphine to be produced and incorporated into the hair. These are all data that I have. Morphine has a much better correlation to inducing than 6-AM or heroin itself. It is kind of interesting, I thought.

Dr. Brown: I have just one question pertaining to the post-mortem result studies. Do you have any thoughts about the extent to which post-mortem results really are a reflection of prior to death levels? I remember a study that suggested that post-mortem results may not be as useful in determining what happened prior to death, particularly with cocaine use as an accidental cause of death.

Dr. Moore: That was from the coroner's report.

Dr. Brown: What are your thoughts about that then?

Dr. Moore: What I thought about it is that the reason that we did this. Post-mortem labs, in general in the U.S., do not analyze hair. The reason that we did this study was, for example, a case where a 16-year old dies of heart failure and the parents need closure that this drug use was a one-time thing and that their child was not a chronic drug user. Hair helps to differentiate between chronic use rather than an acute use. These cases were selected because they were supposedly acute cases, and they wanted to determine if hair provided any more information about longer term use. It seemed to because in almost all the cases there definitely was more than a one time use.

Dr. Huestis: Larry, I wanted to add that too, I thought those were great data. You are right that post-mortem does not really reflect an acute exposure, but it gives tremendous information in interpreting the postmortem specimens because it tells you about history and potential tolerance.

Mr. LoDico: Also I want to comment on those particular data. The use of hair is very appropriate when you have a decomposition of a body and there is no urine or blood. In one of the cases, number 12, there is spleen data, but yet hair yields a wealth of information. You are absolutely right, Dr. Brown, that post-mortem hair represents a global look but does not specifically determine the cause of death.

Dr. Brown: I think that was the point I was trying to make. I appreciate that it represents a source of information. I was somewhat concerned that the persons who reviewed these data are careful in interpreting whether we can endorse the cause of death without an end organ to analyze.

Dr. Moore: The hair results were not part of that decision. The actual case proceeded with the traditional specimens, and the coroner determined the cause of death from them. Hair was after the fact as part of a research project. Those data were not taken into account when the cause of death was determined.

Dr. Brown: Thank you very much.

Dr. Cook: We will now adjourn for lunch. First, I have two quick announcements. For those people who received a Pot Belly menu, please submit your completed form to Phameca. Secondly, will the Board members please meet in the Great Falls room to discuss a few things? And we will reconvene at 1:15. Thank you.

(Luncheon recess)

Methodologies

Dr. Cook: Good afternoon. I hope everyone had a great lunch. We will now to continue with the current perspective of hair testing, beginning with methodologies. Discussing initial testing is Dr. David Engelhart, Laboratory Director of Omega Laboratories.

Initial Testing

Dr. Engelhart: Thank you. I have the daunting task of talking about initial testing of drugs of abuse in hair specimens right after lunch, so this ought to be exciting. Some of the things I will present have already been discussed somewhat. As an overview, I will discuss cutoff levels and target drugs; immunoassays used for hair testing; sample preparation techniques; effective adulterants, such as toxin removal shampoos that are out on the market today; treatments, such as cosmetic treatments; and finally existing standards that are in place.

Shown here are the cutoff levels from the 2004 proposed Guidelines, SOHT, and the European workplace drug testing. Notice that we are in the picogram range for hair versus the nanogram range for urine. Thus, screening techniques must be very sensitive.

Those laboratories with current FDA-cleared screening assays are Omega, Quest Diagnostics, and

Psychemedics. For PCP and marijuana, all have the same cutoff levels. We vary a little bit with the cocaine, opiates, and amphetamines. An issue that needs to be addressed is where those levels should be set.

Though target analytes were already discussed, I think the big difference here is the difference between the target analytes for hair versus urine testing. A good example is cocaine where the target analyte is cocaine versus BZE.

As with urine, the main method to screen for drugs in hair is with immunoassays. What are immunoassays? They are assays which utilize antibodies to identify and measure the amount of drugs or metabolites. They are based on a competitive binding process where labeled and unlabeled drug compete for antibody binding sites. We can compare against known drug standards of known concentrations to obtain quantitative results. The two main types of immunoassays are heterogeneous and homogeneous assays. Heterogeneous assays, of which the main one is enzyme-linked immunosorbent assay (ELISA), are the most common assays used for screening of hair samples today. Advantages of this assay and why we use it for hair testing are that it provides the needed sensitivity in the pictogram range and that it can be very specific. A good example of that is we can differentiate between the d- and l-isomers of methamphetamine with the ELISA assay. They are also not susceptible to matrix effects. Hair is a very dirty sample, not only because of its component proteins but also because of the cosmetic products that can be used. Disadvantages of ELISA include that they are not easily adapted to high speed analyzers, a wash step is required to separate bound and free antigen before a substrate can be added to measure reactivity, and both additional time and labor costs for the laboratory.

The other main type of immunoassay is a homogeneous assay. Advantages include that it does not require that separation step, it can be highly automated, and it provides high throughput and low labor costs. Disadvantages are the sensitivity, specificity, and greater susceptibility to matrix effects. I am not aware of one assay that has the sensitivity to meet all the needs for hair testing at this point.

Whether it is a heterogeneous or a homogeneous assay, the important thing that needs to be remembered about the screening assay is that it must be confirmed by a different technique, such as GC/MS, GC/MS/MS, or liquid chromatography (LC)/MS/MS, which are the most common ones today. This is also a standard forensic practice, to use a confirmation technique that is different from the screening technique.

One of the best ways to demonstrate the effectiveness of different immunoassays is PT programs. There are PT programs in place now that work very effectively to demonstrate the needed sensitivity to meet the detection requirements, to show specificity, to demonstrate precision around the cutoff levels, and to determine whether the cross-reactivity is appropriate. That could come into play with drugs such as opiates where the target analyte is morphine but we are also trying to detect codeine and 6-AM at the same time.

I would like to present some data that show the ability of ELISA assays to meet the required precision, accuracy, and specificity. In this is a study submitted by Omega Laboratories for FDA 510(k) clearances, samples were spiked with cocaine to demonstrate precision around the cutoff levels of 500 pg/mg. In the top table, 11 samples were analyzed for intra-assay precision and were all run on the same day. The coefficients of variation (CV) were all very good. Those 11 samples were also analyzed on 29 consecutive days for a total of 222 results. Again, the CVs were pretty good, all 10 percent or less.

Another aspect of the screening assay is how well does your screening assay agree with what you want to confirm. What a lab does not want to do is subject a lot of specimens to confirmation for no reason. On the other hand, you do not want a lot of false negatives. In this FDA-submitted study, there are 345 samples. Samples with results less than 50 pg/mg were labeled as true negative. We differentiated between half the cutoff and 50 percent above the cutoff, and then samples that were higher than 50 percent above the cutoff. Most samples were in the negative range or in the high positive range. We had very good agreement between the screening result and the confirmation result around the cutoff level.

Here is an example for the carboxy-THC, which is probably the most difficult assay to do for hair testing. We look at the precision around the cutoff level of one pg/mg for intra-assay precision, and the CVs are all very

good at below five percent. The inter-assay precision is also quite good around the one pg/mg range. At higher concentrations, more variation is seen.

Here is also a summary of the agreement between the screening results and the GC/MS confirmation results for 422 samples. Again, most of them lie within the negative range. The other 50 percent are above the cutoff concentration. We do have pretty good agreement around the cutoff of one pg/mg, although not as good as for the cocaine assay. For oxycodone, we had good precision around a cutoff level of 300 pg/mg for both the intra- and inter-assay precisions for 240 samples. We are able to differentiate quite well positives from negatives.

This demonstrates the ability of ELISA. This same type of data would need to be generated with other hair testing immunoassay screening techniques to validate the assay.

Before the sample can be analyzed on an ELISA plate, or whatever the immunoassay may be, the drugs must be removed from the hair via a sample preparation technique. This can be done using buffers; organic solvents; whole hair, cut hair, or powdered hair; enzymatic digestion; or acid or alkaline hydrolysis, which may not be appropriate for all drug classes, such as converting the 6-AM to morphine and then also converting the cocaine to BZE. PT programs that we participate in cover all these techniques used to remove the drugs from the hair. I believe that with the good PT program, you can demonstrate your ability to provide accurate results.

An issue with screening, just like it is in urine, is the effect of adulterants. Tox removal shampoos are available on the internet. If you Google it, tons of hits are obtained. Independent studies have demonstrated that they are not effective at removing significant amounts of drug from the hair. Omega Labs has also submitted studies for their FDA 510(k) clearances. Listed here are the different drug panels that were tested for, the percent positive after the treatment with the toxin-removal shampoo versus before the treatment, and the percent change as measured by the GC/MS confirmation assay. Most of the results are insignificant. Oxycodone had a minus 20 percent, which was the highest one.

Another aspect is the impact of hair treatments, such as hygienic treatments including bleaching, permanents, dyes, relaxers, and so-forth. We also submitted studies for FDA 510(k) clearances evaluating the effects of cosmetic treatments on hair samples. We found these treatments were insignificant on negative specimens. A very limited number of specimens tested positive and then confirmed negative. The effect on the positive specimens was pretty much within the standard uncertainty of the confirmation assay, so it was not a significant effect. This table, showing the effects of the bleaching on hair samples, includes the different panels, the percent positive after the treatment was applied to the samples, and then the percent change. The biggest difference here was with PCP, which showed an average of minus 28 percent. This table displays the effect of permanent treatments on hair, both before and after treatment. The percent positive results after treatment were all greater than 80 percent. Again, the largest effect of any of the treatments was minus 36 percent for PCP. This table shows the effect of dyes on the hair. All of the percent positives after the treatment with hair dyes were 80 percent or greater, and the larger percent difference on average was for oxycodone at minus 19 percent. The last data set was relaxers. Here the effect was not very significant. Percent positive after treatment was 90 percent or greater, except for the carboxy-THC, which showed 67 percent positive after the relaxer treatment. It is interesting that it was only correlated with a minus six percent change on the average on the confirmation test, which suggests that these samples that went from positive to negative after treatment were very close to the cutoff level.

I would like to talk about the existing hair testing standards, techniques that are used in hair testing for screening samples are all scientifically acceptable, and hair tests that are accepted in courts of law. The College of American Pathologists (CAP) now offers hair as a sample in their accreditation process, which covers all aspects of testing in the laboratory, from collection, sample handling, extraction of the drugs from the hair, wash, external contamination procedures, quality control issues, and required PT. There is also accreditation to the ISO IEC 17025 standard and guidelines by the European Workplace Drug Testing and the United Nations.

FDA clearance of laboratory-developed screening assays is also available. To give you an idea of the vast

array of studies that Omega submitted for FDA clearance, there were agreement studies, effects of cosmetic treatments, external contamination, precision, recovery, extraction efficiency, and shipping stability. For shipping stability, samples were placed in front of a heater for a while, shipped then to four different locations all around the country, returned, and finally frozen for a while. A monitoring device that monitored the temperature and humidity the whole time was included. No effect was seen. There were also studies of long-term stability for long-term storage conditions, cross-reactivity, detection limits, and traceability.

I wanted to that that as long as you effectively participate in a good PT program for screening assays, I do not think it really matters what the assay is, whether ELISA, another type of immunoassay, or ELSI/MS/MS.

Dr. Singh: I have a question related to the data that you submitted to the FDA. Are these are all spike studies that were submitted for FDA clearance?

Dr. Engelhart: The only ones that were spiked were for precision.

Dr. Singh: Have you done studies where samples were taken from donors that mimic the concentrations around the cutoffs and that showed similar kinds of precisions?

Dr. Engelhart: We did do precision studies with a limited number of real donor samples. That was part of our submission also.

Dr. Singh: How do that data compare with the spikes studies? Do you see a similar level of precision?

Dr. Engelhart: It was about the same.

Dr. Singh: When you perform the hair preparations, whether digestion or pulverization, I presume you are using the supernatant for the immunoassays. Am I correct?

Dr. Engelhart: You can. It depends on the technique. We do an acidic methanol extraction and the extract is dried down and reconstituted and de-buffered for the ELISA assay.

Dr. Singh: Is everything solubilized in that stage?

Dr. Engelhart: For the immunoassay? Yes.

Dr. Brown: Since I am not an expert on this, I wanted to ask you about the relaxers. Do they all have the same constituents? Are they different? I was curious which relaxers and which constituents you assessed in your presentations.

Dr. Engelhart: I do not have that information with me, but we evaluated two different types of relaxers for the study.

Dr. Brown: Would you be able to provide us with the chemical components of the relaxers?

Dr. Engelhart: Yes

Dr. Cook: Could you provide more details the CAP PT program, including what kind of specimens?

Dr. Engelhart: CAP does not offer PT. They monitor your PT program to make sure you performing acceptably for your accreditation.

Dr. Huestis: Could you provide to the Board the sensitivity, specificity, and efficiency data for all your tables of true positives and true negatives and where they were in the cutoffs?

Dr. Engelhart: No problem.

Dr. Huestis: Thank you. Why do you think PCP was so susceptible in your treatment studies?

Dr. Engelhart: I do not have an answer for that.

Dr. Huestis: One more thing: How do you store your samples for long-term storage?

Dr. Engelhart: They are stored in a controlled environment.

Dr. Huestis: You mentioned freezing at one point.

Dr. Engelhart: That was part of the shipping study that we did. We wanted to simulate conditions that could be in an airplane when the sample is flying across the country.

Dr. Huestis: What exactly is the storage?

Dr. Engelhart: The specimen usually comes in a little manila envelope. We place the sample back in the envelope, put it back in the plastic transport bag, wrap that up, seal it, and then store it at room conditions.

Dr. Huestis: I do not know much about the plastic bag issue. For your samples, you use manila envelopes rather than plastic bags. Did you have any data on that that led you to go with the manila envelope?

Dr. Engelhart: It just maintains the sample a lot better than a plastic bag.

Dr. Cook: Any other questions? Thank you. Our next presenter is Dr. Michael Shaffer, who is Vice President of Laboratory Operations at Psychemedics Corporation. He will be speaking to you about the confirmatory testing methodologies for the hair specimen.

Confirmatory Testing

Dr. Schaffer: Thank you very much for the invitation. My topic today is something near and dear to my heart - MS. I started using MS 1976 when I worked for the Cook County Medical Examiner's Office in Chicago, Illinois; that is where I cut my teeth on MS. We were one of the few laboratories at the medical examiner coroner offices at that time that had a MS. Every time we presented a paper on MS, everyone would get up and leave, saying oh no, another paper on MS. Over the last 30 years, things have changed dramatically. I have spent 14 years conducting hair testing. I also spent some time at Smith-Kline where I was the responsible person managing a NLCP laboratory. I am very familiar with post-mortem, urine, and hair toxicology. Hair has been in use for a very long time. My European colleagues remind me that since they were not mandated to do urine testing, they are flexible. They can test oral fluid, hair, and all kinds of things. So, they have chosen hair testing.

Hair testing is used in a variety of industries, including automotive, oil/petroleum, manufacturing, food services, trucking railroad companies, etc. There is a reason why they use it. It is simply because it provides a 90-day look back window. It yields a quantitative result that hopefully can be reported. With a urine specimen, no quantitative information at all is reported because a result of 115 ng/mL of anything means nothing. With a hair test in which the specimen is washed very well and that wash is evaluated, a quantitative result is obtained that is dose dependent. If you take a drug, it is dose dependent. The amount that is deposited in the hair provides information about how much drug that individual took over 90 days. I do not know how many of you are familiar with the hemoglobin A1c which provides an average over 90 days. It gives information that you did not have before.

There are opinions that I have that may not necessarily be the opinions of other people. Since I am a scientist, I will discuss these with you rationally. But I have great fervor and passion for whatever I do.

I will talk about prerequisites. Obviously, the MS procedure and instrumentation must be validated. But before that, what do you have to do? There are labs that do not wash the hair simply because they never extracted the hair in the first place. When they did wash the hair, they did not obtain much drug. They concluded that you could not wash the hair because the process removed some of the drug. If you do not get the drug out of the hair, then the hair is a worthless test and you cannot use it. That is why recovery studies must be performed and done very well. These studies must be aggressive and have robustness to them. After you get all of the data together, you have to interpret the data. Everything is not black and white, and we will talk about that as well. As far as external validation, that is done not internally but done externally through PT. There are three different PT surveys that we participate in.

Specimen cleanup or preparation is required before MS. This involves removing specimen from the collection envelope, weighing out a portion, making that sample as homogeneous as possible, so there are not short hairs and other hairs. If the sample is not homogeneous, then there may be a challenge; you may have to then do it all over again.

It is mandatory that a wash procedure be used to address external contamination. You need to analyze the wash because a lot of information is derived from analyzing the wash. If you study the washes, you learn that the initial washes are totally different from the final washes. These wash results can be compared to what is inside the hair, thus providing a lot of information. If you do not wash, you do not remove the external contamination, and you do not evaluate it. You cannot make any decisions about it. It falls into a different category, and we will talk about that as well.

The lab must ensure maximum drug recovery from inside the hair, as I explained to you before. If you do not recover well, have 20 percent recovery on a sample, and did not wash, you are measuring the drug deposited on the surface of the hair by sweat. For a level playing field, the lab has to remove all of that. The only way to ensure it has been removed is to measure what is in the last wash. Then you know that you have accomplished it.

There are pre-requisites in the area of quality assurance (QA). The FDA does not clear MS, but they do clear the screening test. The FDA process is very long, laborious, and rugged. They require you to do all kinds of tests, but in the end you are a lot smarter. There is a CAP certification for forensic drug testing in hair. CAP will perform an onsite audit of the lab, using an inspection checklist. They do issue a national certification. There is also an ISO international certification. A number of laboratories have that, we are fortunate enough to have achieved that as well. As part of that certification, we are required to do an annual uncertainty measurement for all of our analytes. We do all of these things to make what we are doing better. If someone visits the lab, they understand what we are doing and how reliable our results are. Quality control measurements with Levy-Jennings (L-J) plots are the bane of all toxicologists. Someone should develop a method of doing it online. I have spent half of my life signing L-J plots. We have a blind external PT program that I started about 10 years ago. Irving Sunshine was the gentleman, scholar, colleague, and friend to whom we sent samples. He sent them back to us and kept tabs with it all this time. That is important as well because it assesses your immunoassay and your MS tests. One of the best things I did when I assumed the job in Culver City, CA was to send the Director of Quality Assurance to John Mitchell's class in North Carolina. The director had no idea before that such a program existed. I have the greatest respect for the program because it is so great.

We have adopted the NLCP as much as possible and practical. There are some Clinical and Laboratory Standards Institute because there is always a regulatory body. There is science and there are regulations. The scientists push for the science, and the regulators hold them back by saying such things as we are really not quite sure you have made that qualitative identification. Obviously, they work together, but you have to know that. Otherwise, if you do not know that, you cannot move forward.

The lab should adhere to the manufacturer's recommendations. Many manufacturers at this time do not recommend tuning and resolution. Therefore, the lab must ask and push them to obtain some information. Manufacturers begin with good laboratory practices, which the lab should obtain from them. The lab should

initially calibrate and tune every day and then extend that to every two days and then three days until you learn what works or does not work and how stable it is. These are all good laboratory practices that every lab should do.

Validation of MS procedures begins initially when the lab is evaluating its method. Is all the drug being removed out of the hair? The first test is to ensure that the extraction procedure, whatever it is, is removing the drug from the hair. Does that take two hours, three hours, six hours, or eight hours? Most of our MS procedures are either six or eight hours. That has been developed over time. It takes a long time, but the lab just has to do it. After you analyze many thousands of samples, you get a better feeling for what it is that you are measuring.

The use of certified standards and controls is obvious. Open controls, L-J plots, deuterated and internal standards are also necessary. No lab in its right mind would not use a deuterated internal standard; you would be foolish not to do that.

Linearity standards studies are performed at five or six different concentrations. From this study you learn how reliable the method is. Because the method will be used every day in the laboratory, it had better be rugged. You do not want L-J plots are all over the place every day.

Precision studies are performed within run, between run, on different days, and with different analysts. It is extraordinarily important to know what the inter-analyst variability is. Everyone must achieve about the same level of aptitude, skill, experience, and so forth. Other studies include limit of detection, limit of quantitation, and upper limit of linearity, which are all simple experiments to do. Also needed are potential interference and potential carryover studies. With LC, almost every inspection checklist requires ion suppression studies. I do not think they are necessary when you use a deuterated internal standard, but that is what they tell us to do so we do it. When I first started in the forensic field, we were fortunate enough to have a MS. If we could, we would do a full scan to obtain a full spectrum for identification purposes. We would also inject a standard on that day under the same conditions and then compare it in the library. That is the best practice that you can do because you cannot do any better than that. What do you do when the concentration is so low that you cannot get a full scan? Alternate techniques are needed, including selected ion monitoring. With a single/quadruple analyzer using electron impact at about 70 electron volts (eV), it is called the hard ionization. Originally, three or four ions were required, but it can be done with two as well. It is important to have a rugged validation package. A hair immunoassay cocaine positive result is confirmed by a MS test. With tandem MS, an instrument that was designed to increase specificity, the hair target analytes are BZE, coca-ethylene, and norcocaine. Toxicologists continue to try to discover another metabolite to increases the specificity. With selected ion monitoring, several ions are measured as they do, and I did, in a urine drug testing laboratory. We had good methods. Solid phase extraction is used to remove it off the column. The concentrations are humongous. The urine is a veritable trap for all kinds of things that come out of you. What are excreted as well are these drugs and their metabolites. If the concentration is too low, then the lab utilizes chemical ionization. Chemical ionization is referred to as soft because it does not use as high electron voltage, usually less than 40 eV. It provides the specificity that it not available with single quad analyzer because it produces a very intense protonated molecular ion, which is used in the instrument to create product ions. Different gasses or chemicals can be used in the second quadrupole to control the reaction to produce different product ions, which are subsequently measured. This increases the sensitivity and the specificity beyond your wildest dreams. We can measure in parts per trillion routinely every day because of a rugged extraction that removed out all the drug, a good clean-up, and the instrument primed to analyze.

We are fortunate to have with us a fellow by the name of Chen Chih Cheng. Though he is no longer with us full-time because he retired, he was with the company for many years. He continues to work with us two days a week even though he receives his full salary. He is the master of MS, which I am not. I have learned a lot from him over the years. I think of myself as a work in progress. I have been a work in progress my entire life because I am willing to learn new things. We learn new things, we change, and we modify; I enjoy and like that.

The tandem MS is what you need for hair analysis because the hair sample size is 10 mg. That equates to 10 uL of a very very dilute urine. Therefore, everything must be working very well. You need a really good team composed of really good people and good methods that are very stable. You must spend an inordinate amount of time on the analysis and the instrument in order to maintain it. It is not a simple matter, but it is all worth it in the end.

Validation of the MS equipment is important because it measures trace levels of drug concentrations in hair. We validate to ensure it is acceptable in the scientific community and acceptable when inspected. If the chromatography is good, then the data are good. I worked in the SAMHSA laboratory in the audit, and they train you very well.

A certifying scientist (CS) must evaluate all these data, including the immunoassay screening data. The CS needs to know everything because if he does not, you have a problem. The CS must know the difference between a negative specimen and a positive specimen. The CS must also recognize when a specimen is contaminated. When is that specimen contaminated? For urine that is adulterated, an interference is found in the sample. The same thing holds true for a hair specimen. You can tell when the sample is contaminated, when it is a very weird sample. You can tell by the metabolic profile and from a number of other manners as well. With an invalid sample, if the donor has done something to his hair that totally destroys it, it will almost fall apart when you pick it. You have must have some idea of what you are doing, what the test is, and the reliability and the accuracy of the test.

In my opinion, tandem MS is needed, that is, GC/MS/MS is required for marijuana and LC/MS/MS is required for cocaine and its metabolites. When we go through those, I will tell you the reason why. Some representative examples of MS protocols are cocaine and its metabolites via LC/MS/MS, carboxy-THC on GC/MS/MS, and amphetamines with LC/MS/MS. I should mention that our wash procedure has appeared in the literature for the past 15 years; it is not a secret, it is not patented, and anybody can use it. Our MS procedure for carboxy-THC was presented at one of the DTAB meetings many years ago. We arrived with our hats in our hands and said we want to help you. That was the most difficult assay, and is still probably the most difficult assay to do. We have also presented twice in the last number of years a cocaine metabolite that I think will separate and provide much better resolution on some topics. We perform amphetamines via LC/MS/MS. Basically, it is LC/MS with an open negative control. In the upper left, there is no peak in this channel, therefore it is negative. These are the deuterated internal standard samples, which show where the d_0, or drug, would be. Deuterated internal standards have deuterium inserted where a hydrogen would normally be. A standard can have two, three, four, five, six, ten, or eleven deuteriums; it all depends on what you would like to do. This slide shows a typical chromatogram. We have these data in table formats. Though this has a scan number of 45, this has a scan number of 87, and this has a scan number of 87, they are in different channels. This is a m/z of 213 while this is 199. This is just a MS chromatogram of cocaine d_0. Looking at the scan, it is 46, which is about the same. Usually what happens is the deuterated internal standard comes out a few scans before. It should always come out before or at the same place. If it ever comes out later, you miss it, which is not good. You must know where these come out by their scan. This is BZE d_0 while this is coca ethylene d_0. These are the deuterated internal standards at five ng concentrations. This is good chromatography, with good separation and good signal to noise. The identification of norcocaine is a little more complicated simply because norcocaine and underivatized BZE share the same channel because they share basically the same molecular weight, and thus they can be confused. For that reason, we do them separately and never together. We perform two injections to make sure that we maximize the testing on that particular sample.

This is something I am still very proud of. When I worked at the Medical Examiner's Office in Chicago, we had some problems with some morphine-containing specimens. A colleague of mine, Nancy B. Wu Chin, and I asked ourselves how we would resolve this. We developed the idea of creating a dual derivative. You make two different compounds, with two different molecular weights, two different m/z, and two different ways of examining it to produce a quantitative result. They all have to agree. We incorporated this, and it works very well.

I presented these data at a meeting several years ago. In this Excel spreadsheet is actual data, not spiked

samples, which we recovered from real specimens. We have a 1.0 pg standard and 0.4 and 1.25 pg controls, which represent 40 percent below and 25 percent above. We also had a couple open negative controls. We are very proud that we had one control at our limit of detection of 0.2 pg. We can detect down to 0.2 on a daily basis, which is fairly remarkable. You have to focus on these things and probably chew out some of the employees quite often to make sure that they are not using old reagents and such. If you just do it, this is what you achieve. We have 474, we have 524, we have dilutions, and we have different areas for the two different internal standards. We work on our program and follow as much as possible the NLCP. Here is an open negative chromatogram containing just noise. This is the 0.4 control with good signal to noise, a nice peak, and not much garbage. We get a 0.334 and a 0.414. These are actually really good data. This is plus 125 percent or 1.25, 1.4, and 1.29, all of which are very good.

This is a chromatogram of a real donor specimen that has gone through the entire procedure, including extraction. These peaks are very nice. We get 0.6 in one and 0.618 in the other. The reproducibility is pretty good. You have to maintain the instruments. We have people that work on them for hours and hours, and then after we go home, somebody else comes in. We have two people that come in at night and wash samples. All they do is wash hair samples all night because it is so very important.

Switching to amphetamines, this is a chromatogram of a calibrator. Calibrator concentrations include a five, a one, and some others. Shown here is a spreadsheet containing all the information about the standards and controls, when they were prepared, when they expire, their concentrations, and all the information that is necessary for the CS to make a decision; it is all made available to everyone. This is amphetamine at five ng per ten milligrams. This is a calibrator. There is the methamphetamine d_0 and methamphetamine d_{11}. We use d_{11} because it gives us a much better response. For Ecstasy and E, shown here is MDA, the metabolite of ecstasy and E, at a concentration at 5 ng. You will never see it at that concentration, but we analyze it. Shown is also MDMA with its internal standard and MDEA with its internal standard. I have never reported a MDEA or an MDMA without an MDA.

There is a group of people, about 10 to 20 percent of a certain group, that pharmacogenomically lacks the ability to metabolize in a certain direction for phase one metabolism. We have seen it with our opiate analysis. Without knowing that the donor has that genetic disorder, the specimen results cannot be reported because there is no MDA. Just as with methamphetamine, we only report it if amphetamine is there. The same holds true for these; we will not report it.

For external validation, there are two options. The SOHT is an international program which sends out PT samples either every four or six months to validate your method. We serve as one of the reference labs for the SOHT. The other hair testing PT program, Arvecon GB, is from Germany and run by Frank Spokard. We also have blind PT program. That is, we make up some drugs, send it off to a scientist, he sends it back to us, he evaluates our results, and he lets us know if we doing okay or not.

I have given Janine a copy of some 50 of our publications which lists all of the methods that we do that have been presented and published, including our washing procedures which are not a secret. We discuss many of the issues as to why you need washing, the rationale for it, and what you usually get out of it.

Conclusions. Sample preparation is critical to MS confirmation. What does that mean? You need to know that the drug can be removed out of the hair. If you cannot remove the drug out of the hair, you may as well go home and call it a day. Recovery studies need to be performed to demonstrate that all the drug can be removed out of the hair. If you do not do that, as my mother would say, finish your food otherwise you do not get dessert. But you have to do these types of things. You have to wash the hair and you have to evaluate the wash to see what is going on. That wash gives you immeasurable amount of information about what was on the hair, what you see, and what was the problem with the first Mandatory Guidelines. There was nothing in there about washing. If you used the Mandatory Guidelines to compare any research, it is going to be dead wrong. You may as well kick it out the door because it is worthless.
Choosing the instrumentation is critical to achieving trace levels of detection. You cannot use a quadrupole analyzer, I have tried. You cannot do it for marijuana, cocaine. And eventually when these other metabolites

come out you'll have to go to tandem mass spec. So pretty much for sure it is marijuana and cocaine. Final analysis, you must consider all relevant data from the entire testing process, not just the MS. You cannot take an MS test in a vacuum. It is not a standalone test. It is everything, all put together. A CS would review everything in the final step.

You also must be able to determine the difference between a negative sample, a positive sample, a sample that is contaminated, which cannot be reported it because it is contaminated. It may be adulterated, or it may be an invalid sample. You must know these things. I work with a very wonderful lady, Virginia Hill, who we call Ginny. She spent her entire life washing hair. She has all kinds of answers; I do not have as many as she does.

A prerequisite to MS instrumentation are procedures for forensically sound hair MS confirmation test. That has never been the issue; you can do it. Everybody can buy the equipment and set it up, so that is not the issue. It is making sure the hair is washed, drug is removed out of the hair, and making sure the playing field is even. Otherwise, it is not an even playing field. Then you have a forensically sound hair test for drugs of abuse. Thank you very much for your attention.

Ms. Farrell: A couple of questions. I spend much of my waking hours now dealing with measurement traceability and uncertainty in many forensic disciplines. Could you educate me more on the certified standards and controls used as part of the requirements for your MS procedure? What is available currently as a true certified reference material that has a reference value, an uncertainty, and measurement traceability?

Dr. Schaffer: We usually only work with certain vendors that supply that type of information. Those vendors provide information that is traceable to National Institute of Standards and Technology (NIST) standards, providing certain reliability. For cocaine, unfortunately, there is not anymore. I think all the rest of the drugs have them.

Ms. Farrell: The certified reference materials are in the matrix already?

Dr. Schaffer: In methanol.

Ms. Farrell: The materials in methanol are then spiked into hair and taken it through your entire process?

Dr. Schaffer: Correct. We also analyze the hair samples that had previously been processed, analyzed, and so forth as controls as part of our quality control (QC) procedure and as part of a blind QC program.

Ms. Farrell: Is the control value based on a historical value or a "certified" value? I want to determine whether we have a good mechanism in this quantitative assay to monitor both precision and bias and whether we have a true reference.

Dr. Schaffer: They are not spiked standards because that would not help us. We take a number of positive cocaine hair samples, a number of positive PCP hair samples, and so forth, and make these samples as homogeneous as possible. We try to homogenize them in such a way that they can go through our procedure and we can get results. Then we analyze the sample about ten times and calculate mean, mode, and other decent statistics. Then that sample is used as a control.

Ms. Farrell: I may come back with some questions on measurement uncertainty. As we examine PT data, it would be very interesting to know how the variation in the population and how a lab's data correspond to its measurement uncertainty.

Dr. Schaffer: You can just come to the lab.

Dr. Singh: Mike, you mentioned an analysis of the wash solution. Please give us an example of how you would do an interpretation of this.

Dr. Schaffer: I can send you 3 or 5 papers, but if you do not mind, I can go through it.

Dr. Singh: Obviously, it is a very critical part of your testing. You said numerous times that you glean a lot of information from the wash. I am curious as to how one goes about analyzing that and should that be the standard for all labs that are in the business of this testing?

Dr. Schaffer: I cannot force any lab to do anything, I can just say what we do.

Dr. Singh: From the perspective of the Board, we need to understand what it is that one is dealing with.

Dr. Schaffer: For the first Stout paper, they sent samples contaminated with pharmaceutical cocaine. Pharmaceutical cocaine contains about one percent cocaethylene. Previously, it was thought that cocaethylene could only be produced by transesterification when the user drank alcohol and so forth. I do not know if anybody sells pharmaceutical cocaine on the street, but it s theoretically possible to obtain it. What is gleaned from the wash is the fact that you cannot remove it all, and it remains in such a concentration that it provides information that we use in our analysis to tell us if the sample has been overly contaminated. For example, if the last wash of a hair sample contains two nanograms of cocaine, it does not meet our criteria for reporting. We multiply that concentration of cocaine that is in the last wash by five, which is equivalent to washing continually for five more hours. This is a benefit of the doubt to the donor in that respect. Usually, last wash concentrations are in the range of 2, 1.7, 1.6, and so forth. So, 2 times 5 is 10. Unless that value is 15 or above, we cannot support that sample as positive. Sometimes we obtain negative numbers, which implies that there is something awry with the sample.

Dr. Wong: Mike, you mentioned that the CAP offers a survey.

Dr. Schaffer: The CAP does not offer a survey for hair. They accredit laboratories, and then CAP expects the laboratories to enroll in a survey. Last time we were inspected, CAP cited us for not having a PT for the pain management drugs – oxycodone, oxymorphone, hydrocodone, and hydromorphone. Because we are very proactive, we had five years' worth of positives in our archives. If you want to know what the history of five years' worth of positives, we will analyze those for some money.

Dr. Wong: There is no CAP survey program for screening and confirmation?

Dr. Schaffer: No. The SOHT and the Germans work together to offer PT programs. The German one is a very good one.

Dr. Huestis: Could you explain how you chose the amphetamine of one, the methamphetamine of five, and that ratio? Could you provide the number of specimens that are positive for methamphetamine but had no amphetamine so we could look at that data?

Dr. Schaffer: I am not sure I understood the question.

Dr. Huestis: You told us that you do not report out methamphetamine without amphetamine, and you had methamphetamine at five and amphetamine at one.

Dr. Schaffer: Those are the standards, and we also have other standards at other concentrations. I am sorry for the confusion. There was also five as well.

Dr. Huestis: You do not require a one fifth concentration?

Dr. Schaffer: No. The cutoff for reporting amphetamine is 0.5.

Dr. Huestis: You require amphetamine just to be present, and not at a ratio?

Dr. Schaffer: The ratios we have seen after wash are totally different from the ratios that appear in the literature when a wash is not done.

Participant: 0.5 is equivalent to 50 pg/mg.

Dr. Huestis: How do you handle the issue of chirality? Do you analyze for d- or l-isomers?

Dr. Schaffer: It is very interesting. If you know the cross-reactivities of your d- and l-isomers in your immunoassay and create a curve, at a cutoff of five there is a nice response with a value of about 53 percent. Taking that to the MS, and the MS is five. With another sample, it is about 50s with the MS at 100. A little bulb goes off. The cross reactivity is so much poorer for the l-isomer. A concentration of about 100 is needed to give the equivalent of a five. In a way you can tell, but you cannot know for sure until you do the d- and l-isomers. Did you follow me?

Dr. Huestis: You perform the d- and l-isomers?

Dr. Schaffer: We have performed about 1000 of them, with only two of them being l-isomers. You can easily pick them out by examining the data. I believe the l-isomers were a result of a bad chemist who was on drugs or something when he performed the synthesis.

Dr. Huestis: Going back to how you determine the amphetamine concentration, was it based on analytical capability or experience with thousands of samples? How many specimens have methamphetamine but no amphetamine?

Dr. Schaffer: There are no such specimens. You will not be able to detect a methamphetamine concentration of one because it is very, very low.

Dr. Huestis: There are some?

Dr. Schaffer: There would not be any because it is below the sensitivity of your method.

Dr. Huestis: How did you choose the amphetamine cutoff? Is it analytical capability?

Dr. Schaffer: If you have a five and it is 10 percent, five and 0.5 sound like good numbers.

Dr. Huestis: You picked it out of whatever?

Dr. Schaffer: From somewhere. If you look for it, it should be there. If you do not find it, then you have a problem.

Dr. Cook: Thank you Mike. We are running out of time for questions.

Dr. Huestis: I have one more question. You referred to a cocaine metabolite that you thought would be definitive for intake, but you did not name it.

Dr. Schaffer: Mahmoud ElSohly was kind enough to synthesize for us meta-, para-, and ortho-hydroxycocaine. The FBI and the Medical College of Virginia visited our lab, and hopefully the FBI will do something soon. Someone told me she was confident that she could tell if it was ingestion or contamination just by these values.

Dr. Cook: Our next speaker is Dr. Jeri Ropero-Miller, who will speak to you about proficiency testing for the hair specimen.

Proficiency Testing

Dr. Jeri Ropero-Miller: Good afternoon. In almost every single presentation today, PT was mentioned. So I could just say “ditto” and sit down. The people who talked about it provided the conclusions of PT testing, so I will tell you the how and why.

For this presentation, the two PT programs that I am summarizing are the NLCP hair pilot PT program that transpired from 2000 to 2007 and the SOHT PT program that has been ongoing from 1995 to present. I will end with a summary table, which includes three other PT programs. These are not the only PT programs that exist, but these are the ones that publish their findings in the literature so that you can know how the laboratories are performing. Because, as many people have said, PT testing is that external QC for the laboratory, so they can test themselves against other procedures, determine whether their results are what they think they are, and whether they are implementing corrective action to resolve the issues that they have in their laboratory.

In April 1997, DTAB had two meetings to discuss drug testing in alternative matrices. From that, it was decided that a pilot PT program would be started. In 2000, we began sending out the first PT samples. During this time, up to 13 laboratories participated in the PT program, and there were 23 cycles of PT over this eight year period. The first eight cycles were more or less method optimization, with us trying to get our bearings. Did we want to powder the hair? Did we want to leave it in strands? Did we want to provide drug user hair? Did we want to use fortified spiked hair samples? Did we want to standardize the methods or did we want to leave it up to the laboratories to use the methods that they were using in their everyday analysis? In the next three cycles, cycles nine through 11, we focused on the stability of the PT samples. At that point, we had decided that to use both drug user hair and fortified spiked PT samples that we produced at RTI. We wanted to evaluate the stability of those samples over a six month period and to assess the variability within and between laboratories. The last 12 cycles of this pilot PT program focused on the inter- and intra-lab variability, and that is what I will present today. During this whole pilot PT program for the NLCP, one of the most important findings was that there still remained large variation in the results that were being reported by the laboratories, and that is why the last 12 cycles focused on both inter- and intra-laboratory variability. For these last 12 cycles that took place in 2006 and 2007, we originally had eight laboratories indicating that they wanted to enroll in this study. The labs agreed to receive their results, and if they had any issues, to diligently work to correct those issues to proceed forward. Two laboratories enrolled late, and one laboratory left during that 12-month period. At any given time, there were six to seven laboratories participating. With some of the data, all of the analytes were reported by all the laboratories. Remember, we were focusing on accuracy and precision. The other thing I wanted to stress in this is these were single PT produced samples. We created them, aliquoted them out, stored them ambient temperature, and every four months, we would send out the next round of PT samples at the specified time. This involved confirmatory testing only because we were evaluating variation and determining how the laboratories were doing. The other thing that was unique with these PT samples is we asked the laboratories not to decontaminate the samples because we decontaminated them as part of our PT program. We asked the laboratories to start from that step forward. The samples were all authentic hair, and they were either drug user hair or fortified hair samples. There were PT samples in our earlier cycles that were drug-free hair, but for these particular samples, because we were analyzing them for a certain analyte, all were positive for a directed analyte.

There were three samples sent to the laboratories over a year, beginning at time-point zero with 0 months, 3 months, 6 months, and 12 months. All of the concentrations for our fortified hair samples were spiked at a target concentration to be either one and a half to three times the 2004 proposed confirmatory test cutoff concentrations. The laboratories were directed to analyze each analyte five times in one batch over different days. These PTs were multiple batches, over multiple days, with results being 20 analyses of that specific PT sample.

Set one was our THC and amphetamine sample. We had four samples in this PT step. The first two were with the THCA. The first one was a drug user’s hair, so concentrations were higher than the proposed cutoff. The reference laboratory concentration was 0.82 pg/mg. Our second THCA sample was at three times the proposed cutoff at 0.15 pg/mg. We had two amphetamine PT samples that had amphetamine,

methamphetamine, MDA, MDEA, and MDMA at one and a half and three times the confirmatory cutoff.

The second set was our cocaine PT. The first sample was a drug user's hair, so the concentrations for cocaine and BZE were quite a bit higher. The coca-ethylene and norcocaine were right at or slightly above the proposed cutoff concentrations. The second one was at one and a half times. The third sample had cocaine only, and it was at three times the cutoff concentration or 1500 pg/mg. We also had a BZE-only sample that was at one and a half times.

The final set that was our opiates and PCP set. We had one drug-user hair and three fortified hair samples. We had three opiate samples and one PCP at one and a half times the proposed confirmatory cutoff concentration. Remember, the laboratories did not decontaminate the sample but went straight to confirmatory testing.

For laboratories' methodology, we did not ask the laboratories to provide details on how they performed their analysis. We did include a sheet that summarized the analytical procedures in use. From this, I have amassed together what information we had but certainly not in as much detail as some of the other PT programs have published. All the laboratories used deuterated internal standards for the targeted analyte. For instance, they would not include deuterated methamphetamine to quantitate amphetamine. Not all analytes were reported by all laboratories.

Some of the laboratories chose to powder their hair strands because that is the process they used in their laboratory, other laboratories left their strands intact, and still other laboratories cut their strands up into smaller, more homogenous, samples.

As far as pre-treatment techniques, there was a gamut of what was used, from no treatment at all, to either water alone or an aqueous buffer, to organic solvents, including methanol, methylene chloride, acetone, or those solvents altogether. The laboratories could use one or multiple washing steps; we did not know the details. We did know that some labs did nothing, some did water only, aqueous only, solvent only, and some did a mixture. All digestion techniques were used by the laboratories, which included no treatment, acidic or basic depending on the analyte of interest, and enzymatic. We asked the laboratories to only list the digestion technique. Thus, we knew it was enzymatic, but we did not know the enzyme source. For extraction techniques, both liquid-liquid and solid-phase extractions were used by the laboratories.

For quantification and identification, the analytical techniques used by the laboratories were all mass-spectral techniques. Predominantly, GCMS, either with electron ionization or with chemical ionization, was employed. Some laboratories used GC/GC/MS or tandem mass spec for their analysis. They did report which method they used.

At the end of each of the PT cycles, the NLCP reported back to each of the laboratories their results along with the group results, so that the labs could assess their results and how they fared. At the end of each cycle, we held a web meeting with all the participating laboratories to review the results so that they could understand the results and comparisons.

This table represents the laboratory PT results for amphetamines and THCA. In each column is the analyte with the targeted concentration in parenthesis. Listed down each column is each of the cycles with the mean concentration for all the laboratories. At the end of each column are the means plus or minus the standard deviations. Looking across those columns, pairing the targeted concentrated and the mean concentration of all the laboratories, notice that the labs are doing well at hitting near the targeted concentration for these particular analytes. I can show similar data for PCP, the opiates, and cocaine as well. Thus, these data indicate that over this the one year period, the PT samples that we produced are stable.

The next three figures are interaction plots, displaying laboratory results versus the PT cycle. The y axis has a mean concentration measured in pg/mg while the x axis is each of the laboratories. In this particular figure is shown a methamphetamine PT sample targeted at 900 pg/mg. Shown here is the first cycle, or cycle 12, for this PT set. For the first cycle, there was much variability from lab to lab, as depicted across the x axis and

following that particular line. Over the course of one year, the inter-laboratory variability, as demonstrated by cycle 21, did seem to improve for this particular PT sample and for many of the analytes. For example, laboratories G and M had very good intra-laboratory variabilities; their concentrations from cycle to cycle were very consistent. This interaction plot is for cocaine with a PT sample at one and a half times the confirmatory cutoff concentration. Notice the variability among the laboratories. One laboratory that may have a higher concentration than all the laboratories for one analyte or one cycle, but that may not be necessarily seen for the next analyte or the next cycle. Again, laboratory G was the best performing laboratory for this particular PT cycle. Looking at their percent CV for this example, it was about five percent. Even laboratory L, which had greater spread in their results, had a percent CV just over 10 percent for cocaine. The last interaction plot is for a THCA PT sample at three times the cutoff level. There were three laboratories reporting on this particular sample. Laboratory G again seemed to be performing the best for this particular PT sample. An ANOVA analysis of these interaction plots at a p value less than 0.05 found a significant difference between the laboratories. This indicated that the variability among laboratories was not what needed to be improved.

This slide depicts another way of examining these data. This bar graph gives the system variation over a one year cycle. For this particular bar graph of the amphetamine analytes, on the right-hand side right is THCA in the last two. For each of the analytes are the zero month results or cycle one, followed by cycle two results, cycle three results, and cycle four results. The last bar for each of the analytes, shown in the yellow or on far right, is the urine PT results that were received in both the maintenance PT program and in a special study that was ongoing about the same time, 2004-2005, for the methylenedioxyamphetamine analogs that had not yet been put into the maintenance PT program. Looking at a percent CV of 10 percent that is acceptable for urine, in all cases, the urine CVs were at 10 percent or less. Unfortunately, for the hair, CVs were quite a bit above that. MDEA and methamphetamine were the only two analytes that started approaching a percent CV of 10 percent.

This bar graph provides similar information on the other analytes that were not in the previous bar graphs. Included are opiates and PCP and on the far right is BZE. The hair PT results are shown in the darker blue on the far left, the pilot PT results for oral fluid are in the center, and then the last bar represents the urine PT analytes from the NLCP maintenance program. In comparison to oral fluid and urine, hair still has some work to be done to get those percent CVs down to where we would like them to be.

In summary, we were able to demonstrate with this study that we had stable materials for all the drugs over a one year period. The results for the participating labs were not consistent at that time. Some of the analytes had an increase in variability over their one year period. This, we felt, was illustrating that laboratories had a challenge in trying to maintain their performance over a long period of time on the same sample. The materials performed well using the analytical methods that were chosen by the laboratories at that time.

The SOHT PT Program is a long-term, ongoing society PT program, ongoing since 1995, and offering two PT samples a year. Their program does offer both qualitative and quantitative results. What is nice is they post their PT results on their website for anybody to view and compare. Per Jurado and Sachs in an article published in Forensic Science International in 2003, the SOHT developed their PT program so that all laboratories that perform hair analysis can produce comparable results or at least detect the same compounds. It is a voluntary program for the laboratories just as the NLCP pilot PT program was a voluntary program. The number of participating laboratories in their PT rounds at any given time range from 18 to as many as 36. Their first PT report was published in 1995, followed by other papers in 1997 and 2001. On their website, biannual results from 2007 are posted. From 2008 to 2010, they prepared a special PT with ethyl glucuronide.

Today, I am presenting two years of data. One is their 2001 data and the other is from the Jurado and Sachs paper of 2003. The goals of the PT program were to evaluate the laboratories' accuracy of detection, quantification, and the performance of the different extraction methods. The laboratories provided a lot more detail about their extraction methods, but I will not discuss that today. It is a good paper to learn of the extraction methods that were in use in 2001. For this particular PT round, 15 laboratories and 3 reference labs participated. The reference labs used by SOHT do not participate in their PT program, so they are 18 separate laboratory results. The reference concentrations are determined by the reference laboratories, which perform

10 analyses on each of the aliquots. Those results are summed together to obtain the reference concentration. All of their samples are short hair segments, and they use both drug user hair and drug-free hair. For this particular PT round, three samples were shipped out, including drug-user hair containing cocaine, amphetamines, and THC; another drug-user sample containing cocaine and the opiate dihydrocodeine; and a drug-free hair. Very similar to us, not all of the analytes were reported by all laboratories. Though 15 laboratories participated, 8 laboratories did not report amphetamine results, and 9 did not report cannabis results. For the qualitative results from this PT round, there were one false negative for BZE, 6-AM, and morphine; three false negative results for codeine; and one false positive result for codeine and 6-AM. In reporting their quantitative results and examining the mean inter-laboratory percent CVs reported in the paper, their percent CVs were higher than the NLCP results. They reported CVs between 42 and 71 percent for all analytes except THC; that one jumped up to 93 percent. Back in 2001, they still needed to do some work too. The authors and the labs participating in the PT program felt that the extraction procedures used by the laboratories and the variation in the methods used by the laboratories were the major sources of their variation.

Fast-forwarding 10 years for this program, in 2011 their ongoing goals were the accuracy in detection and quantification. In this 2011 PT round, there were 36 laboratories participating and 3 reference laboratories. The PT samples were similar: drug-user hair containing cocaine, opiates, and THC; another drug-user hair containing cocaine, methamphetamine, and THC; and one drug-free hair sample.

When SHT reports their sample precision data, they focus on z scores rather than percent CVs, but they still report percent CV. The z score is calculated by subtracting the overall mean from the participating laboratory's mean and dividing that by the difference between the 75^{th} quartile and the 25^{th}. Per the SOHT guidelines, a PT result will be satisfactory if the z score is less than or equal to 2.0, questionable if the z score is between two and three, and unsatisfactory if the z score is greater than three. For this 2011 round for 6-AM and morphine, one or two labs did not analyze these analytes, and there was one questionable result out of 36 laboratories. For codeine and THC, however, there are more laboratories either not analyzing or reporting negative results. Codeine had 13 no results and one unsatisfactory result, whereas THC had 10 laboratories that either did not analyze or reported a negative result and one laboratory with unsatisfactory results. For the cocaine sample, one lab did not analyze and two reported questionable results. BZE had four laboratories that did not report. Methamphetamine had seven laboratories that did not analyze or reported negative results and one laboratory with questionable results. The mean inter-laboratory percent CVs in 2011 are 34 to 55 percent, except for codeine, which was 306 percent. This was attributed to one laboratory reporting a really high concentration for codeine. When the SOHT has a new laboratory in the program, their results are incorporated with all the results. It is unknown whether it is a new laboratory reporting those falsely high or falsely low numbers.

Besides the NLCP and SOHT, there are also three other PT programs: the German Society of Toxicological and Forensic Chemistry (GTFCh), HAIRVEQ, and French Society of Analytical Toxicology (FSTA). GTFCh has had literature published since the late 1990s, and they still provide PT samples as a fee for service; online, a lab can register for PT samples and participate in that program. HAIRVEQ is another published PT program from Germany and Spain; their two publications were in 2004 and 2006. The FSTA did most of their PT samples in the early to late 1990s and have not had published in the last 10 years or so.

This last table summarizes those five PT programs in comparison with the NLCP pilot PT program. The PT programs are listed in the first column. The second column lists those PT programs using authentic hair samples. Some of the PT programs use only drug user hair, whereas others are using spiked or fortified hair and drug users hair, like the NLCP has done. For the analytes, most of the same analytes are being analyzed. Exceptions include the PCP analyte being analyzed in the NLCP program only. For THC, the NLCP reports the THCA, whereas other PT programs analyze either the parent THC or cannabidiol. Whether fortified hair samples or drug user samples, concentrations are either well above the proposed cutoff concentrations or are higher than those used in the NLCP. Whether hair washing is performed is left up to the laboratories in most cases; if hair washing occurs, most laboratories do not report the specifics. Some programs provide powdered PT hair samples, as does the GTFC and the HAIRVEQ. In most cases, the PT program lets the laboratory decide what methods they will use for preparation and analytical methods. Two programs, the HAIRVEQ and SFTA, had at least one or two rounds where they used standardized methods. They provided an exact

procedure that the laboratories were supposed to employ. Though the results from this standardized protocol may have shown improvement, it was a small program and it was unknown whether the same laboratories participated or not. Though the results were slightly better, CVs were still greater than the 10 percent.

Our conclusions are as follows. PT testing is a necessary QC measure. Results for the NLCP and other programs demonstrate that the participating laboratories are not providing results that are currently consistent. Inter-laboratory and intra-laboratory precision results remain higher than other testing matrices. The PT materials seem to be stable, whether authentic hair samples or fortified hair samples. Finally, the laboratory preparation and analytical treatment of the hair contribute to the variablities of the PT results. Shown here is a list of the references I used for this presentation.

Dr. Cook: In the interest of time, there will be no questions now. Our next speaker is Dr. Jim Bourland, the Scientific Director of Alere Toxicology. He will provide you with some of his own personal experiences with hair testing.

Best Practices Experience

Dr. Bourland: Good afternoon. My name is Jim Bourland. The focus of my talk is drawn from my experience with hair testing from about five years ago. From 1997 to 2007, I was intimately involved with a Las Vegas hair testing laboratory, Associated Pathologists (APL), which is now Quest Diagnostics. Even though Janine mentioned that you will hear best practices from a laboratory that is doing hair testing currently, Alere does perform hair testing in this country. I cannot comment about the hair testing that Alere does across the Atlantic because I am not familiar with that. I will discuss my experience with hair testing and its practical aspects for those of you considering laboratory hair testing, especially those aspects you need to think about and the required investment. I will progress through the process, starting with collection, what a collection looks like, the accessioning process, the pre-analytical process and its challenges, extraction, screening, and confirmation. I will share a few older studies, including a paired hair and urine study with 7000 specimens that I presented in 2002. The specimens were collected from the same donors, so it is apples to apples.

For the collection process, hair is collected from the back part of the head, either the occipital crown region or the posterior vertex. Typically, it is cut with scissors in two to three locations in the back part of the head and cut as close to the scalp as possible. It is done in such a way as to try not to affect the cosmetic appearance of the person and to do the least amount of damage so that the donor does not have big bald patches in the back of his head. As Peter mentioned, hair is not pulled. Hair testing is often called hair follicle testing, but that is an incorrect term. We are not testing the hair follicle but rather the mature hair above the scalp. Once collected, the collector aligns the root ends of hair in a foil packet.

If someone does not have head hair, what do you do? Obviously, body hair is an option. It depends on the laboratory, the protocol, and the collection as far as what body hair to go to next. The order is typically armpit hair, arm hair, and leg hair. If hair from these sites is not available, hair from other body areas, including pubic, is used. We received all types of samples when I worked for Quest Diagnostics. If you do analyze body hair, the interpretation is much different because a longer period of time is represented. Also, it is less specific in what time it really does represent. Thank goodness that body hair does not grow like the hair on the back of our head. So body hair is an option. If the person has weaves or short head hair, these are also challenges.

The collection process, just like any collection process, is an important aspect in hair testing. During the collection process, the donor may claim that the person cutting his hair contaminated it with the scissors or whatever. Marc LeBeau gave a presentation at the American Academy of Forensic Sciences about the variability of cutting from the scalp. What time span is represented if certain collectors cut to get as close to the scalp as possible? Does this weigh into the interpretation once the hair is analyzed?

Shown here is a typical hair collection kit. Typically, an alcohol pad, such as an isopropyl alcohol swab, is used to clean the scissors. The cut hair is placed in this foil packet and aligned. The foil packet is folded and then placed in this envelope. Imprinted on the envelope is a ruler. This convenient ruler is used in the laboratory

accessioning department by the accessioner to measure the first 3.9 centimeters from the root end of the hair. It is this portion that is then tested. The test request and chain of custody forms are filled out; the cardboard envelope is initialed, dated, and sealed; and then everything is placed in a plastic bag for shipment to the laboratory. Once the plastic bag is opened in the laboratory, it is discarded. What is stored is the three by five cardboard envelope. The lab can store a large number of specimens in a small amount of space. Special refrigerators or freezers are not needed because the hair specimens can be stored at room temperature over long periods of time. That is briefly the collection process.

Once the hair is collected, it is sent to the laboratory. If the laboratory does other types of testing and is not exclusively a hair testing laboratory, then a sorting process is required. If the lab is also performing urine or oral fluid testing, the hair specimens are sorted out because they all undergo different processes.

Then the accessioning process takes place. The major task in the accession process, besides opening the envelope, is cutting and weighing the hair to prepare it for the pre-analytical process. It is ideal and probably necessary to have a very isolated, separate, and clean environment to perform this accessioning. Each accessioning station will typically have scissors, a cleaning solution for the scissors, something to wipe that off, an accurate scale, a Sharpie, and some sort of disposable pad for each specimen or means to clean the area between each weighing. The accessioner takes one specimen envelope at a time, opens the envelope, and then aligns that bundle of hair to the ruler, measures it to 3.9 cm (1.5 in), cuts that bundle of hair at the 3.9 cm mark, takes that first 3.9 cm section, cuts that into further smaller pieces, mixes it for homogeneity, and then weighs out either 10-20 mg, depending on your procedure in the laboratory and whether a screening assay is performed. We originally started with 20 mg, but I think they analyze 10 mg now. The accurately weighed hair is placed in a test tube for the pre-analytical process. During accessioning, accession labels are generated just as it is done in a urine drug testing lab where scanning bar code and split tube labels are created. The test tube, cardboard envelope, and test request form are labeled with matching labels. Per the forensic process, one specimen at a time is accessioned before proceeding to the next one. This process takes much longer than it does for either urine or oral fluid specimen. It requires a commitment if the lab decides to do this type of testing.

One of the things that Peter mentioned, but you may not think about, is how hair is typically 30 percent water by weight. Some sort of humidity control in the space where hair accessioning is occurring is important. Las Vegas, for instance, is very dry; the generated static electricity causes hair to fly all over the place. The lab must be cognizant of this and try to control for that as much as possible.

How is hair aliquoted? As mentioned previously, the hair is cut 3.9 cm from the root end and then is cut into smaller segments. Though some of the labs powder the hair, we did not do that but rather cut it in smaller segments, mixed it up, and weighed it. The first aliquot was for an ELISA screen for the five SAMHSA drugs, and the second aliquot was for GC/MS or GC/MS/MS confirmation.

After accessioning, the specimen is placed in a test tube for screening or for confirmation. As Peter so eloquently showed, hair is a very difficult matrix. It is not a homogeneous matrix or a liquid matrix. I am not surprised when comparing the CVs of urine and oral fluid to a solid matrix. Also, the labs are using different extraction techniques with different recoveries of the drug from this heterogeneous solid matrix. It is no surprise to me that better CVs are obtained when everything is perfectly mixed in a solution versus this solid matrix in which something must be done to extract the drug out of the hair. Not only is a pre-analytical process required for the confirmation but also for the screening assay. Unlike urine, the hair specimen cannot simply be aliquoted and placed on a chemistry analyzer. Some sort of extraction, digestion, or whatever procedure is performed to prepare it for the immunoassay. Typically, the process involves a solvent, an aqueous solution, or a combination extraction. The extract is dried and reconstituted in a liquid that is acceptable for ELISA before performing the screening assay. A pre-analytical process is required prior to extracting for GC/MS, GC/MS/MS, LC/MS/MS, or GC/GC/MS, which is sometimes used as a Dean switch technique for added sensitivity.

The pre-analytical process should also include a wash procedure to remove potential external contamination, not just from drugs, but also from gels, dyes, or other things you may not even think about that could interfere

with the ELISA test.

There are two main approaches to this pre-analytical process to remove the drug from the hair. One is the digestion of the hair into a liquid. The solid matrix is converted as much as possible into a liquid. Many of the digestion processes are not quite 100 percent efficient. Sometimes a mixture of solid and liquid occurs, so the lab must work with that. Another approach is to subject the hair to acid or base, solvent, or a mixture of those to dissolve the hair and extract the drug from the liquid rather than digesting it. The challenges associated with a digestion technique are that many drugs are instable, especially cocaine for example. A sodium hydroxide digestion is not used for cocaine because it will be destroyed. A balance act is required to keep the drug intact. With an acid, some of the 6-AM is converted into morphine, another factor to consider. All these factors must be remembered when performing this pre-analytical process. Then the lab must balance between the efficiency of the extraction and the turnaround time. Do you have six to eight hours or only two hours to do the extraction process? The laboratory must consider these in this process.

This information is from a talk that I gave comparing hair and urine with some of the pre-analytical processes that were used in 2002 for the confirmation techniques. For cocaine and amphetamines, the pre-analytical process involved four washes followed by dilute acid and heat to extract the cocaine. For the opiates, a methanol buffer and potassium hydroxide digestion was used for a partial ingestion of the hair. PCP involved a simple methanol and heat treatment, the same extraction used for the screening; additionally, acid could be included as well. For THC and THCA, hair digestion was mandatory to elute THCA from the hair. Next, all analytes are subjected to solid phase extraction. From here on out, the rest of the analytical process is similar to what is done in the toxicology laboratory for other matrices such as urine or oral fluid. These have to be balanced with the amount of drug present in the hair. Besides the challenge of the hair matrix, analytical sensitivity is required. The lab can perform solid-phase extraction, liquid-liquid extraction, derivatization, deuterated internal standards, calibration curves, and QC. As Mike covered in detail, the same process that you would use for any kind of toxicology testing is done once you reach to this point. There are many confirmation technologies available, including single quadrupole, GC/MS, and GC/GC/MS with a Dean's switch. The GC/GC/MS does enhance capability and can potentially be used in this application.

For THCA, I think one of the best methods is GC tandem MS and possibly LC tandem MS as well. The lab must utilize a hyphenated technique for THCA. The lab will definitely have a more robust method if that technique is used for cocaine and metabolites as well. Classic GC/MS is acceptable for four of the five SAMHSA drugs, excluding THCA. It may be difficult to imagine with pg concentrations. With a cutoff, for example, of 300 pg/mg and using 20 mg of weighed out hair, 20 times 300 is 6000 or six ng. A one mL aliquot of urine would equate to a six ng/mL cutoff. That is a challenge, but it can be done with standard GC/MS.

In the interest of time, I will skip through some of this. The majority of hair testing, when I was involved in it, was performed primarily by three laboratories, Psychemedics, Quest Diagnostics, and Omega Laboratories. Psychemedics was the pioneer and the originator of hair testing. At the time, they were by far the leader in terms of sheer volume of specimens analyzed every day. I do not know what the numbers are now and which lab does more or less, but all three laboratories are very active in hair testing. The screening assays for these three laboratories are FDA 510(k) cleared. Though I do not mean to leave out the other hair testing labs, these are the only ones that I am either directly or indirectly familiar with how they perform hair testing.

I want to make a comment about the marijuana metabolite. Christine mentioned performing THC parent screening and then confirming for THCA. She has a point. The 1 pg/mg does refer to the carboxy metabolite, which is used as the standard. Based on the pre-analytical process used, at least in our hands and my experience, THCA is not being extracted. THC parent is extracted which then cross-reacts enough with that antibody to produce a reaction. THC parent is from 50 -100 times more concentrated than the metabolite in hair. Whether you call it carboxy or THC, the THC provides the response.

The cutoffs are similar, but a little different, between the labs. This chart, shown in Quest colors to make Barry happy, is from the Quest Diagnostics website. For Quest, PCP has been dropped, but the expanded opiates have been added since I was there. I do not know whether the inclusion of PCP is something the Board needs

to consider. The cutoffs for amphetamines and cocaine are at 300 pg/mg and the opiates cutoffs are at 500 pg/mg. Omega is reversed, with a 300 pg/mg cutoff for opiates and 500 pg/mg cutoffs for the amphetamines and cocaine. Confirmatory levels are also a little different.

Now I will discuss some interesting data that I presented at an American Academy meeting in 2002. The data were collected from January to July of 2001 from clients that submitted both urine and hair at the same time. In other words, the subject would provide both hair and urine specimens on the same visit. It is a paired study; therefore, we are not comparing one population to another population. These data will hopefully add to what was already shared. I know Barry has more recent data than this. This is all non-regulated testing. Though I will use the word SAMHSA because I tested for the SAMHSA drugs, the specimens are all non-regulated. Our urine panel at the time included THC, cocaine, amphetamines, opiates, and PCP. We also screened for barbiturates and ethanol, but those data are not included. For hair, the same five SAMHSA drugs were tested, but the amphetamines screen used an amphetamine/MDMA antibody. When referring to amphetamines, I am referring mostly to methamphetamine. I did not exclude the amphetamine-only positives in the data. There are 7207 paired samples, so about 7000 of each for urine and hair both. There were different reasons for the test. NM means not mentioned, indicating that the reason for the test was not recorded on the test request form. NR means not recorded, which is the same as NM. OT means other, indicating another reason, at 7.7 percent. PA refers to post-accident, PE is pre-employment, RA is random, and RC is reasonable cause or reasonable suspicion. By far, the most frequent reason for the test was pre-employment. Remember, this was kind of a different population. Some random tests and some other tests had higher positivity rates. In this population, there is probably a higher positivity rate than found in the general pre-employment population.

For marijuana, 2.8 percent of these 7000 specimens were positive for the marijuana metabolite in both the paired hair and the urine samples. In other words, the hair and the urine from the same subject in 2.8 percent were positive. In another 2.3 percent of the population, the hair was positive but the urine was negative for marijuana metabolites. These would have been missed with a urine test. In 1.2 percent of the population, the urine was positive for marijuana metabolite and the hair was reported as not detected. The total overall positivity rate for marijuana in hair plus urine is 5.1 percent. In the same population, the total positivity rate in urine was four percent, which though less, is not significantly different. There are reasons for that which I will discuss at the conclusion.

For amphetamines, a little different pattern is seen. 2.4 percent of the population had paired positive results in hair and urine. 4.6 percent were positive in hair for methamphetamine mostly and some amphetamine only but negative in the urine. 0.3 percent had a positive result in the urine but negative in hair. There were very few samples that were positive in the urine only for amphetamines. If you are positive in urine, you are also most likely positive in hair as well. The overall the positivity rate in hair versus urine in the same population was greater than two times that of urine. The hair positivity rate was 6.1 percent versus a 2.7 percent in urine for the same population of individuals.

For the cocaine metabolite, the positive rate was 1.2 percent in both paired hair and urine. In hair, the cocaine metabolite, the cocaine parent, and other metabolites are present. In 3.8 percent of the population, the hair was cocaine positive, but the urine was negative for the cocaine metabolite. Thus, there was no positive BZE in the urine for 3.8 percent of the population. In 0.04 percent of the population was found a urine positive result without finding a positive cocaine hair sample. If a donor was positive in urine for cocaine, then he was also most likely positive in the hair test as well. The overall positivity rate for cocaine in this population versus the positivity rate of cocaine in urine was about four times greater in hair. Many more of the cocaine users are detected with the hair test than with the urine test.

We did analyze for opiates and PCP, but there was not a significant number to present.

Overall 6.5 percent of the population was positive in both hair and urine. 10.6 percent was positive in hair but negative in urine. 3.1 percent had a positive result in urine but negative in hair. Overall, 17 percent of the hair specimens were positive for at least one drug or metabolite compared to 9.6 percent of those that were positive for at least one drug or metabolite in urine. There is about a twofold increase in detecting positives in

hair versus urine.

Why are there more dramatic differences in cocaine? Pharmacokinetics explains much of that. Cocaine has a fairly rapid half-life and appears in the urine early and is detectable for 48 to 72 hours, or two or three days, typically. With hair, the detection window is three months, so historical use is detected. That is probably why more dramatic differences are seen.

Marijuana is detectable much longer in urine. The differences between the time windows will not be as significant. Also, the marijuana metabolite does require accumulation from repeated use to become positive in the hair. Marijuana used one time is unlikely to be positive in hair. Conversely, cocaine or methamphetamine used once or twice in a 90 day period may possibly produce a positive result. With marijuana in hair, the frequent to chronic users are being identified because of frequency of use. Obviously, a combination of both matrices yields the highest detection rate.

Today, as it was in 1997 when I first started hair testing, there were the two main roadblocks to interpreting hair testing results. One is the issue of external or environmental contamination. What do you do about that? The other one is hair color bias. Is it use or exposure? There are many donor claims of exposure, especially with marijuana and cocaine, the main two drugs that people claim they were exposed to.

I want to examine at external contamination in a little different way. When I was at American Medical Laboratory (AML), we had a unique client base. Child protective services agencies would request hair testing from children that were exposed to environments where their parents or caregivers were clandestinely involved in manufacturing drugs or were very heavy drug users. In a study, we compared hair results from these children with hair results from the adults in the same environment to assess the difference between exposure versus drug use.

For methamphetamine, we had a request to test 117 hair specimens from children who were allegedly exposed to methamphetamine. 80 of these specimens had methamphetamine only detected with no amphetamine, the metabolite, whatsoever found. Based on our reporting criteria at the time, these would not have been reported out as positive results. Three of the specimens were amphetamine only. In 14 specimens, methamphetamine was detected, but the amphetamine result was less than 50 pg/mg, thus not achieving our criteria for a positive methamphetamine result. That left 20 specimens that met out reporting criteria in these children that were exposed. I examined those 20 specimens in greater detail. Two of the 20 specimens were from infants - a five month old and seven month old. What does the hair on a five or seven month old really represent? Could this be a prenatal exposure event? Could this be through breastfeeding? How could the child get amphetamine and metabolite in their system as well? For 15 of these specimens, the children were ages one through five with eight of them being two year olds. Children at this age are in the toddler stage, with lots of crawling and hand to mouth action. If it is a very dirty environment , as it exists in a clandestine laboratory with drugs powdered all over the place, is the child putting his hand in his mouth and actually passively ingesting the drug? Could that be why they presented as positive? There was one 7 year old, an 11 year old, and a 12 year old. Could they actually be taking the drug or just exposed? I do not know. For the adults in the same time, the same number of specimens was analyzed for comparisons sake. Of the amphetamines that went to confirmation, 110 of the 117 specimens met the positive reporting criteria while seven specimens did not.

I wanted to determine whether we could glean information from the overall ratio. Looking at the mean concentrations and the range, there are overlaps. I do not know how much you can really take from this. But obviously, the mean concentration in the adults was much higher than the children for methamphetamine. The amphetamine in children is either not there or at a very low ratio. The average ratio of methamphetamine to amphetamine is 36:1 in children and 10:1 in adults. This is a graphic representation of that, with the children shown in the purple bar and adults in blue for methamphetamine and amphetamine.

We did the same thing with THC or marijuana. There were 126 requests for directed analysis of THC. Now, we did something unique at APL, AML, and Quest at the time. We looked for the THC parent and THC metabolite.

We did that routinely initially, utilizing two extractions and two methods to report the parent and the metabolite. Then we stopped doing it routinely and only did it for these special cases; eventually, we stopped doing it all together. I collected all these data before this was turned off because I thought it was valuable to see if there was any kind of pattern with these children. Of the 126 requests we had, 104 specimens were positive for THC. The mean age of these children was 4.15 years and ranged anywhere from three months to 16 years old. 94 specimens out of 104 had THC only with no marijuana metabolite whatsoever. One specimen, however, had THCA present above our cutoff at the time, but the THC parent was present below the reporting cutoff. In four specimens, THC was detected but the marijuana metabolite was below the reporting cutoff. That left five specimens that met our reporting criteria for both the THC and metabolite present for a positive result. Two of the specimens were from teenagers - a 14 year old male and 16 year old female. Could this represent drug use? That is a good possibility at that age if they are in that environment. The other three specimens were from children ages one through three. These were a little unusual, or as I thought, very interesting. They had very high THC concentrations and fairly low THCA, but technically, they are above the cutoff. Is it passive inhalation of marijuana smoke or is it due to the deposit on the outside? I do not know, but the data are interesting to look at and think about. Shown here are the adult data. Notice the dramatic difference in the ratio again between the children and adults. THCA is present from zero to very little in adults on average. There is much more THC in hair than THCA.

There are all kinds of questions that get asked of a hair testing lab. I have projected some of these. These are not all the questions I received. Some of the excuses I cannot discuss here as far as why a donor has a positive result, but I am sure you have heard them all. There were many interpretation questions, providing many things to consider. Hair testing has utility, but there are limitations on the interpretation of the results. We need to really consider these. With that, I will end my presentation.

Dr. Cook: Though we are running behind, people are getting antsy so we will take a five minute break. (Break) I want to assure the public commenters that we will get to you. We are running late, but we have had some really good presentations. I wanted everybody see these data because I believe it is important. Our last speaker today is Dr. Barry Sample who is Director of Science and Technology Employer Solutions for Quest Diagnostics. He will present some hair testing data. We are again having technical difficulties. The presentation is viewing sideways, so our remote attendees may not be able to see this properly.

Hair Drug Testing Data

Dr. Sample: Thank you Janine. It is a pleasure being here to talk with everyone about the data. There are some slides that I will flip through very quickly. These are more background that the Board might be interested in in terms of methodology that was used. I really want to take the time to focus on the data. There are two things that I want to talk about. One is paired hair and urine drug test results that were from simultaneously collected drug and hair specimens. The other is our workplace data, which is part of the Drug Testing Index. From that, I will discuss some selected drugs and testing reasons.

You have seen this slide before, which shows the differences between urine, oral fluid, and hair testing. For the paired data, the goals of the study were to compare the positive prevalence rates for those simultaneously collected hair and urine specimens and to look at the concordance between those specimens. While these data are primarily workplace-related data, included are also criminal justice, Family Social Services, and Child Protective Services data. For the purposes of looking at paired data, I think it is still applicable; it does not necessarily matter whether it is all workplace data or not. About 193,000 specimens were collected between January 2004 and June of 2009 for this study. Because of the large number of workplace specimens, 73 percent of these specimens were pre-employment, 12 percent were random, and 15 percent were other, which relates, to a large extend, to that child and protective services type of testing. For the Board's information, the methodologies for both the urine and the hair screening are shown here.

This first slide shows the overall prevalence rates. I will spend a little time explaining how these data are laid out, and then the data will make sense as we go through the other drugs. On the top of the table are the positive prevalence rates combined over that 4.5 year time period. The overall positivity for hair specimens was

12.6 percent while the overall positivity for the urine specimens was 7.6 percent, a 66 percent difference. In other words, there are 66 percent more positives in hair than in urine. For the concordance tables, specimens that are positive in hair and urine are in the upper left-hand quadrant, specimens that are positive in hair and negative in urine are in the lower left-hand quadrant, specimens that are negative in hair and positive in urine in the upper right-hand quadrant, and specimens that are negative in both specimen types are in the lower right-hand quadrant. The top number, not in parenthesis, represents the positive prevalence rate. For example, looking in the upper left-hand quadrant, the positivity rate for specimens that were positive in both hair and urine was 4.7 percent. 85 percent of the specimens were negative in both hair and urine. The data in parenthesis is the percentage of the positive specimens in one or both specimen types that would fall into each category. From an overall perspective, 30.6 percent of the positives were positive in both hair and urine, 51 percent of the positives were positive in hair and negative in urine, and 18.4 percent of the positive specimens were positive in urine and negative in hair.

Before examining the positivity rates for amphetamines, we need to discuss the assays. For urine testing, there is one immunoassay that detects relatively equally both amphetamine and methamphetamine. The hair ELISA assays are targeted against methamphetamine, so a presumptive positive is unlikely in a hair specimen unless there is a tremendous amount of amphetamine only there because the assay is primarily detecting methamphetamine. The hair positivity rate for amphetamines was 5.9 percent while the urine positivity rate was 2.1 percent, which is a 179 percent difference. This equates to nearly two and a half more positives in hair than in urine for amphetamines as a class. Hair does a great job of detecting amphetamines, accounting for essentially 90 percent of all the positives. Thus, a hair test will uncover 90 percent of those amphetamines positives while a urine test going will detect about 33 percent of those specimens. Because this is almost an apples and oranges comparison, let us examine just the amphetamine data where the differences become even more dramatic. There was a 222 percent difference in positivity rates for amphetamine in hair as compared with urine. If all you did was a hair test, you would find 94 percent of the methamphetamine positives. There were only approximately six percent of the specimens that were positive in urine and negative in hair. If all you did was a urine test, you would find about 30 percent of the methamphetamine positives. These results are similar to some of the data presented by Drs. Schafer and Bourland earlier.

For cocaine, the differences are even more dramatic in these paired samples. A 635 percent difference in prevalence was found, with hair at 4.8 percent and urine at 0.65 percent. If all one was doing was a hair test, 98 percent of the cocaine positives would be found. If all one was performing was a urine test, about 13 percent of the positives are found. Only 2.1 percent of the specimens were positive in urine and negative in hair. This is consistent with what you have heard earlier but very dramatic differences.

With the other drugs, a different pattern is seen. With marijuana, America’s favorite illegal drug, there is essentially no difference between hair and urine overall positive prevalence rates. For the concordance data, about third of the specimens are positive in hair only, roughly a third of the specimens are positive in urine only, and a third of the specimens are positive in hair and urine. No matter which specimen type selected for marijuana, you will miss approximately a third of the specimens that are positive for that drug if that is the only test performed.

Opiates tell a slightly different picture. Caveats for these results are that the data for opiates are a little skewed. In the urine data, some was screened against at a 2000 cutoff while others were screened against a 300 cutoff. Testing for prescription opiates, the so-called expanded opiates panel, was not quite as prevalent in this time-frame, but some of these urine data did include testing for those prescription opiates as compared with the hair testing that is only looked at codeine, morphine, and 6-AM. Thus, these data are a little harder to interpret. In this paired data set with those caveats, there was a 56 percent difference in prevalence rates with more positives detected with the urine test than with the hair test. Per the concordance tables, if all one was doing was a hair test, you would detect about 35 percent of the positives. If all one was doing was a urine test, you would find about 85 percent of the positives. These differences may, in part, be attributable to cutoff. If we have learned anything from the recent cutoff changes, it is all about cutoff in terms of detection capabilities. The fact that urine also included prescription opiates would tend to also impact this data. Therefore, I would not over-interpret those differences.

For PCP, I would call it a tie, with a one percent difference between the two. Very low prevalence rates are found. It is similar to marijuana, with about a third of the specimens positive in urine only, a third positive in hair only, and a third positive in both specimens.

Next, we will examine our workplace data derived from our Drug Testing Index, which Quest has been publishing as a resource to government, industry, and the media. This is our 25th year now of publishing these data. The source for these data is all workplace testing. We go to great pains to exclude data from rehab clinics and criminal justice testing done via a chain of custody. We also try to exclude data from those clients that are performing their own screening, whether it is lab-based screening or point of collection testing, and only sending us confirmations; these specimens would be high positivity samples and would tend to skew the data. These are all laboratory positive data prior to MRO review, thus it is not known whether there is an alternative medical explanation. The dataset also includes any employer or MRO blinds. Considering that blinds are primarily a federally-mandated testing phenomenon and that 80 percent of those blinds will be negative, for the larger prevalence drugs, blinds will have much less of an impact. There are two major groups that comprise this dataset. One is the federally-mandated safety-sensitive workforce and the other is the U.S. general workforce, which includes all private sector company policy testing that is not being done per a government mandate, such as with DOT testing. I will also weave in some data from the National Survey of Drug Use and Health (NSDUH). This is an annual survey of non-institutionalized individuals, approximately 67,000 people 12 years of age or older, who are interviewed for their self-reported use. Thus, you can compare our workplace data with the NSDUH report.

For overall positivity rates in urine drug testing data, there have been some dramatic declines, particularly in the general workforce, since we began publishing these data. In 1988, the overall positivity rate in the combined U.S. workforce was over 13 percent. Since the late 1990s, these rates have been relatively constant and have essentially leveled off. In the federally-mandated data, the jump seen here in 1994 is related to THC screening cutoff change from 100 to 50 ng/mL. The data do not reflect the opiate change in 1998. We are seeing this decline in positivity rates in our urine drug tests.

We should now focus on the general workforce data because is most similar to the hair data because it is non-mandated testing. In our hair data, overall positivity rates have somewhat declined since 2003, which is not much different than what we saw in the general workforce urine data. In more recent years, the decreases in those positivity rates are more evident. While there have been some ups and downs in hair positivity rates, it is certainly down compared with where it was in 2003 and 2004. Currently, the overall positivity rate is around six or eight percent in hair as compared with four percent in urine.

This NSDUH data represent those respondents who are in the workforce. There are two groups shown here - those respondents who work for an employer that has no drug testing program shown in blue and those respondents who work for an employer that has some sort of drug testing program, whether pre-employment, current employees, or both. There is there roughly a 50 percent higher self-reported use of an illicit drug in the previous 30 days in those whose employer has a drug testing program. These data suggest that drug testing works as a deterrent in keeping drug users out of the workforce. If you look at our data, whether it is urine or hair, with the declines that we have seen over time, particularly early on in those programs, it would suggest not necessarily that drug use among workers is going down, but rather that drug use among those employees subject to testing is decreasing. These data are reinforcing the deterrent effect of drug-testing.

Examining the percentage of test results by testing reason in the U.S. general workforce, the number of random tests has essentially doubled between 1997 and 2012, from roughly 8 to 16 percent in that timeframe. Certainly, the percentage of pre-employment tests might be impacted by the downturn in the economy. If you examine these data with an economic lens, some of those declines are coinciding. That being said, there is still an overall increase in random testing. For the pre-employment testing, there has been a slow gradual decline in the positivity rates since 2007. Of interest, in 2012 there was an increase in positivity rates in the U.S. general workforce back up to a level not seen since 2007. The data for federal testing are not surprising. In 2011, the first full year of the lowered cutoffs, there were 30 percent more cocaine positives and roughly the

same increase in amphetamine positives in that time period. The data for random testing, particularly in the general workforce, is similar in that it has been declining since 1997. Data for our hair test are a little harder to examine. While there have been some ups and downs in the pre-employment positives, at best it may be slightly downturn if you do some eyeball averaging and take that long longitudinal view. Our random testing positivity rates have, even with all the ups and downs, shown some declines in recent years.

Examining results by drug category, for the urine amphetamines class, which includes amphetamine and methamphetamine, there have been some dramatic increases in recent years, year over year increases, in amphetamines positives. That we believe, based on discussions with MROs, is primarily driven by increasing prescriptions for Adderall. Focusing just on methamphetamines, there was a big uptick in methamphetamine positives in 2003-2004, which mirrored the explosion of methamphetamine labs around the country, followed by a rapid decline and then a holding steady of methamphetamine positives. For amphetamines as a class, the urine data are driven by amphetamine positives and not methamphetamine positives, which have been holding steady over the last couple years. For our hair testing data, ignoring the percentages, those data are very similar from a trend perspective.

Beginning in 2007, we tracked some tremendous declines in urine cocaine positives, absent the cutoff changes and the federally-mandated workforce. 2011 was the first full year in both the federal and general workforce for the urine cutoff changes. Interestingly, in 2011 and 2012, federal positivity rates for cocaine were higher than those in general workforce for the first time since 1997 or for as long as we have been tracking these data. In the hair data, there may be somewhat of a lag in that cocaine decline, with the larger decrease in the hair positives for cocaine occurring in 2008 or 2009. The patterns are similar with hair and urine tending to track very closely with one another. Per the NSDUH data, self-reported cocaine use declined in 2007-2008, with bigger declines occurring in 2009 through 2011. Holding steady was that big difference, roughly 50 percent more positives, between those respondents that work for an employer without a drug testing program as opposed to those that do. Comparing the NSDUH, hair, and urine data, the trends are tracking relatively uniformly across all those data sets.

Marijuana, which is the last drug in the workplace dataset that I will discuss, has shown some declines of late. The trend has been holding steady for the last three or four years in general workplace positives for marijuana at about two percent. That trend is not necessarily seen in the hair data. Though the 2012 hair data dropped dramatically from 2011, one year does not a trend make. Taking the long view, there have been some ups and downs, with 2012 being not much different than 2005 or 2009. It is a little too early to tell what is going on there. Per the NSDUH data, particularly among the respondents that work for an employer with a drug testing program, the self-reported use seems to be holding steady. Perhaps that means people taking a urine test are doing a better job of studying for it or using a wider variety of the products that are sold to tamper with the tests. Those strategies, as we heard earlier, are much less effective with a hair test.

To summarize, the paired data positive prevalence rates for some, but not all, of the analytes are higher in hair than in urine. Both specimen types are complimentary. If you are only doing one drug test, you will not necessarily find all of the positives. A test for recent use, such as urine or oral fluid test, and a test for repetitive long-term use, such as a hair test, could provide a more complete picture of an individual's drug usage. Our workplace data show the positivity rates for employers who drug test has declined. NSDUH certainly suggests that drug testing helps deter drug users from, at least, those employers who have such a program. From the cocaine data, lower cutoffs, no matter what specimen type is, would detect more drug use. Our Drug Testing Index data, at least for amphetamines and cocaines, mirrors the U.S. population data.

Dr. Cook: We now come to the public comment period. I will make sure that everyone who wants to give public comment has an opportunity, even though we are running behind. Nine people have registered to give public comment. We will begin with those eight that are here in person, followed by the one who is joining by teleconference. To begin is Robert Bard, who is Managing Director of Healthcare Technologies Consultants. If you please, come up to the microphone.

Mr. Bonds: Can we obtain a transcript of all the public comments?

Dr. Cook: Yes. The transcripts are published on the website, and then they are abbreviated in the minutes.

Public Comments

Mr. Bard: This is my third time speaking to the Board in the public session, and I would like to do this again. Today's session is the first time we have actually talked about hair testing, and I congratulate the Board for at least having the opportunity to have people discuss it. I would like to point out some of the observations from the last group after lunch, in that what we have seen are laboratories, mainly the larger laboratories, the three that have been identified, having set up compliance systems that both have regulatory and other standards applied to their systems. You have heard CAP, the FDA 510(k) processes, CLIA certification, and the ISO 17025 standard for laboratories being used. You have seen laboratories with well-valued systems in place. I draw your attention to some of the observations made for the PTs where data suggested that the laboratories are not doing very well. Maybe the laboratories that have been chosen for these PTs have been the wrong types of laboratories. I would hope that the Board would request copies of PTs from the laboratories that we have identified, which were Psychemedics, Omega, and Quest. Possibly, there might be one more that would be in the large group. Take the opportunity to see if they would provide you with PTs, so you could get a better understanding of their performances. Let us take a look at hair testing in these laboratories.

Hair testing today is legally admissible in court and has been since about 1995. If you take the Daubert evidence cases and apply the Fried's rules, you will find that that this is acceptable information. We have not arguing that this should not be allowed, we are arguing that SAMHSA should advance the process to accept this as an alternate method.

Considering the states and their inability to decipher what the appropriate methods are, they opt for doing nothing or refusing to allow alternate testing of any type. That is unacceptable. It makes the transportation industry do double testing for no reason at all. You have a method that is amiable and capable of testing far in excess of what you have with the gold standard of urine. As was presented by most of the laboratories, testing has been equal or better in most cases.

Let us examine where we are at. In 2004, the Mandatory Guidelines provided for alternative matrices. In 2008, they were stripped out completely, and only urine was allowed in. That is where we remain today. The Mandatory Guidelines are virtually unchanged since 2004. We are not going anywhere. We are still waiting for oral fluid testing, which is probably a couple years off. If we consider oral fluid testing timeline, we would not see hair testing for another five to ten years.

Dr. Cook: I want to remind everyone that the Board will not comment in the open session, but we do take it under consideration in the close session. The next public commenter onsite is Lane Kidd, who is the President of the Arkansas Trucking Association.

Mr. Kidd: Thank you very much. I am not a scientist; my major is in communications. I can tell by looking at your faces in the audience that you are ready to go home. Thank you for this opportunity. I have about six to seven minutes worth of comments. My remarks are more practical and political in nature, and they are more in view of the marketplace as it relates to drug testing, as opposed to most of the presentations you have heard.

I am offering remarks on behalf of two organizations. The first is the Arkansas Trucking Association, which represents about 300 businesses and private fleets, including FedEx Freight, JB Hunt Transport, Wal-Mart Transportation, and other major fleets. I am also representing a group in D.C. called the Alliance for Driver Safety and Security, commonly referred to as the Trucking Alliance. It represents seven major trucking companies, two of which are among the largest 10 trucking companies in the U.S. The Trucking Alliance was formed in 2010 because several major trucking companies believed there were specific trucking industry safety initiatives that if adopted by Congress or at the federal level would vastly improve highway safety, not only for the trucking industry, but for the motoring public. The first major issue that we took on was one that had been in the regulatory channels at the Federal Motor Carrier Safety Administration for about 10 years, and that was

to create a drug and alcohol clearing house. Since the 1930s, our three million truck drivers account for their hours of service operating a vehicle by filling out a paper logbook. Devices are available on the market today that, if installed in a commercial truck, would verify the number of hours that a driver operates his truck. We simply promoted and supported legislation that would mandate that those devices be installed in every commercial truck in the U.S. This bill had been flowing its way through the governmental annals of bureaucracy for 10 years. The stars aligned for us last year when Congress passed the Transportation Bill, which included this mandate. In two years, there will be a mandate whereby every commercial truck in the U.S. will have a device installed that will verify how many hours a truck driver has been operating his vehicle. We think that is a good law.

Our second initiative was to mandate electronic on-board recorders. This initiative relates to drug testing in the industry in support a drug-free workplace. We support drug-free truck drivers. We are one of the few industries in the U.S. that operates literally within feet of millions of people every day. It is incumbent upon us as an industry to make certain that very driver behind the wheel of an 80,000 pound tractor trailer rig is not using drugs. To illustrate the practical application of why we, as an industry, support hair testing as an option in lieu of urine testing is to tell one story. The two trucking companies involved are both members of the Arkansas Trucking Association. Three years ago, a young man applied for a position in a trucking company as a truck driver. The interview and application process went well, and the trucking company conditionally offered the driver employment subject to a urine drug test. The driver took the urine drug test and passed it. This particular company, as a matter of corporate policy, required a second drug test, a hair test. The driver agreed to the hair test, it was conducted, and it was positive for cocaine use. The trucking company said thank you for your application, but we cannot hire you. So, the truck driver applicant went on his way. Three weeks later, the same person showed up at a second trucking company. Again, the interview process went well. He was offered conditional employment as a truck driver subject to a urine drug test, which is the only federally-recognized method for determining drug use in the trucking industry. This company conducted that test, and the driver passed the test with a negative result. He was hired as a truck driver, delivering freight primarily in the western states. Time passed. It was around midnight when a Colorado policemen west of Denver spotted a tractor trailer rig going westbound in the eastbound lane of I-70. The trooper gave pursuit for nine heart pounding miles but could not get the truck driver to stop. Then the inevitable happened. The tractor trailer plowed into an oncoming car, killing the motorist, a 71 year old grandfather, who to his credit steered his car in a certain way so that his granddaughter in the backseat lived. In the post-accident test, the truck driver was found to be positive for cocaine use. It was the same truck driver as I just told you in the previous story. Now, this created a basic flaw in the system. Under our current rules, the second trucking company had no idea that the applicant had tested positive at the first trucking company. That is why two U.S. senators, one of whom is U.S. Senator Mark Pryor from Arkansas, and the late Senator Frank Lautenberg, introduced legislation two years ago that would create a national drug and alcohol clearing house. Anyone who tests positive on a pre-employment test for drug use will be listed in this database. Congress, in its wisdom, included this clearing house in the Transportation Bill last summer. By next year, this clearing house will be created. Every trucking company before it hires an applicant will query the database to see if this person ever tested positive at another trucking company. We believe this is a good, common-sense database. There is a second problem. Under current rules and laws, the results for the applicant who tested positive on his hair test will not be permitted into the drug and alcohol clearing house because the only test results that will be allowed in the clearing house will be from the urine test. We potentially have thousands of people who will test negative on a urine test but positive on a hair test who will be able to drive a truck. For that reason, we are supporting legislation this year that will soon be introduced that will direct HHS to promulgate rules allowing hair testing for truck drivers only on pre-employment exams only as an option for trucking companies. If a trucking company chooses to use hair tests in lieu of a urine test, then that trucking company will be permitted to do so and will not be required to conduct two tests as they are today, costing our industry millions of dollars and also allowing drug users to skirt the system. All of us today agree we do not need truck drivers passing urine tests but failing hair tests to be permitted to go to work. Thank you very much.

Dr. Cook: Our next public commenter is Raymond Kubacki, who is CEO of Psychemedics Corporation.

Mr. Kubacki: My name is Ray Kubacki, and I am CEO of Psychemedics Corporation. You have a very

important job ahead of you as you take a fresh look at hair analysis, and we all recognize that. It is important that you be provided with a balanced perspective of where the industry has been, and more importantly, where it is today. I have been CEO of Psychemedics for 22 years, so I can provide perspective on that as well, provide some balance to what was really a very unbalanced first presentation this morning on hair testing, and tell the rest of the story.

There are a couple of major points to keep in mind. Number one is that there has been a broad acceptance in industry and in the workplace for hair testing. There are a significant number of Fortune 500 companies from a wide cross-section of U.S. business, including transportation, petrochemical, automotive, casino services, etc. There are other companies besides Psychemedics who are performing drug testing now using hair analysis. It is been widely accepted over these 25 years. There are quite a few people relying on hair testing right now as you sit and begin to deliberate. These clients have some very significant hard data, some of which has been shared with you already and some of it will be shared by our clients. It is paired data and not data from different pools which would provide a distorted view. The second point I want to make is that hair testing has been broadly accepted in the courts. To try to cite a pending civil service case and not to talk about all the federal, administrative, and state cases where drug testing has been upheld over the last 25 years gives a very unbalanced perspective. These include four federal court cases where some of these issues in these other cases have been adjudicated. We at Psychemedics would be very happy to work with you in any way that we can.

The one thing I would also suggest and hope is that when you adjourn for two days and in future days to study this is to bring in some of the experts who have actually been doing drug testing. We, Dr. Schaffer, and others would be very happy to answer questions. We have quite a bit of experience that we are happy to share with you.

Again, as you heard from the last speakers and some of the ones that will come up here now, the stakes are very high. Because one thing we do share is all of us are on the highways. All of us have our loved ones on the highways. It is very important job that you have. We will help in any way that you would like. Thank you.

Dr. Cook: Our next public presenter is Don Osterberg, who is Senior Vice President of Safety, Driver Training, and Security for Schneider National from Titletown, USA.

Mr. Osterberg: Thank you very much for the opportunity. I learned a tremendous amount about hair today. I did not know prior to today that hair can physically hurt, but during some of those technical discussions my hair was actually hurting me, but I got through it. The last two speakers have framed this in the appropriate way. It is fundamental in my view that illicit drug use among commercial drivers be stopped. Truck driving is a safety-sensitive vocation that shares our workplace with the motoring public. We have an obligation because this is a public safety issue. As a result, it deserves expedited processing and not a lethargic process in reviewing the benefits associated with hair testing among commercial drivers.

Let me set the context from a national perspective. The most recent reports indicate that last year 3,887 people died in truck-involved crashes in this country. That normalizes to nearly 11 deaths every day. If you look at a longer run average of 10 years, that number is over 4,500 truck-involved fatalities in this country, or nearly 13 deaths every day. As a result of that, we have to do everything that we can.

What really raised my awareness, back in 2007, was a study I read that was conducted by the Oregon State Police Forensic Services Division. They anonymously collected urine samples from 487 commercial drivers. They only had a four percent refusal rate, which I thought was pretty impressive. Of those 487 samples, there were 41 positives for a positivity rate of 9.65 percent in urine that was tested for drugs. I was alarmed by that number. I went back and determined at our rate of positive tests on pre-employment drug tests. Looking at results from the five years prior, the positive rate ranged from 0.20 to 0.29 percent. If you think about that, a pre-employment screen for commercial truckers is prescheduled for the pre-employment drug tests. There are, in my view, two conditions under which an individual would fail a urine-based drug test. Either they are so hopelessly addicted to drugs that they cannot abstain for 48 hours in order to pass the urine-based test, or they

are intellectually challenged. No one should fail a pre-employment urine based drug test because you know when the test will be collected. I compared the delta between our positive rate on pre-employment urine testing and the Oregon State police study. We asked ourselves a question: What is a better way to detect chronic use of drugs among safety professionals like commercial drivers? In conversations and in benchmarking with counterparts in the industry, I learned of hair testing.

I want to share with you some numbers. First of all, prior to implementing our hair testing program, our post-accident positive drug test rate was 3.06 percent. Put into context, that was roughly six post-accident positive drug tests per year. After implementing hair testing for pre-employment screening, we had less than one per year for the first three years, and we have not had a single post-accident positive drug test in the last three years.

Let me share some other numbers. We have now conducted 50,951 paired tests. When the driver goes to a clinic, we take both urine and hair specimens, which we send off to the respective labs for testing. Of the 50,951 tested, 1,813 were positive on the hair test. 153 of those 1,813 were also positive on urine. The good news is the delta. There are 660 commercial drivers who are not driving a truck today for Schneider National. To the point that Lane Kidd made, they are undoubtedly driving a truck for someone else on the highways shared with our families. As safety professionals, we believe that is completely unacceptable. As a result of that, our obligation is to use the best possible testing protocol to identify chronic drug users in our workforce. We have zero tolerance for drugs in our safety-sensitive vocation.

I applaud the Board for taking on this issue, but I would ask that you examine it through, first of all, the lens that this is a public safety issue. Look at it pragmatically; though hair testing is not perfect, it is better at identifying drug use in this particular demographic. My short answer to that is yes, and I would ask that you expedite the review process and recognize hair testing as a viable alternative to urine testing for the vocation of commercial trucking. Thank you.

Dr. Cook: Our next public commenter is Abigail Potter, Research Analyst for the American Trucking Associations.

Ms. Potter: I have an expanded version of my comments with references to studies that I have examined which I will distribute. My name is Abigail Potter, and I am with the American Trucking Associations (ATA). ATA is a united federation of motor carriers, state trucking associations, and national trucking conferences, created to promote and protect the interest of the trucking industry. Directly and through our affiliate organizations, ATA encompasses every type and class of motor carrier operations.

First, I was very excited to read the FR today, and I am probably the only one. We have been supporting the eCCF for a long time. It will be very important in speeding up the process to determine whether there has been a positive test. Speeding up the process for pre-employment is something that is needed with our industry.

Don took my title for my comments, which is Hair Testing: Perfect No, But Better Yes. Hair testing to detect illegal use has gained increased popularity worldwide. This is especially true within the private sector due to the testing method's superior ability to detect illegal drug use. In fact, dozens of major companies have adopted hair testing, including Kraft, Shell Oil, Toyota, Michelin, Whirlpool, and British Petroleum. However, despite widespread use and major advances in hair testing methodology, misconceptions about its limitations persist. These misperceptions are often based on outdated understandings of the testing process and time-worn research studies. Improvements in the testing process and more recent research findings have addressed these limitations. Accordingly, it is now appropriate for public policy makers to revisit the efficacy and the benefits of hair testing. Hair testing labs have made significant strides in distinguishing positive results due to environmental contamination from those attributed to illegal drug use. New testing methods are capable of distinguishing active use of the drug from its environmental contamination.

Historically, as we have heard today, critics of hair testing have pointed to the fact that the FBI laboratory no longer conducts tests to detect cocaine use involving individuals who have a legitimate reason for being in

contact for cocaine, for example, law enforcement officers that handle drug evidence. Though this is a valid concern if individuals have a legitimate exposure to high concentrations of environmental contamination, for the overwhelming majority of test subjects, this is not the case. As the researchers Fritz Prast, Hans Sach, and Pascal Klintz have pointed out, it is quite unlikely that an innocent citizen in his daily environment might contaminate his hair to such an extent to cause a cocaine positive result according to the criteria. After 30 years of successful experience with hair analysis, hair testing continues to be a suitable tool in the majority of application fields, including testing for cocaine exposure.

The other primary concern about hair testing is the presumption that the test process is systematically biased against individuals with higher levels of melanin in their hair, especially for people with darker hair. However, researchers have repeatedly evaluated this contention and have found little or no statistical evidence to support it. In 2011, the Transportation Research Board published a synthesis of research on drug and alcohol testing in the truck and bus industries, including a review of alternative specimens. The authors evaluated 15 articles related to the effect of melanin levels and race on their hair testing results, and none of the reviewed articles found direct support of the race bias hypothesis.

Most researchers would agree that hair testing, like all specimens, has some flaws and limitations. However, the question is not whether these flaws are more pervasive than the limitations of other specimens like urine, especially knowing urine's greater potential for subversion. However, there is also a general consensus that hair testing is a superior means of detecting prior drug use, particularly in a context of pre-employment and random tests. Adoption of hair testing should not be hindered by unsupported claims or an outdated understanding of the testing process. Hair testing has been widely adopted worldwide, and it is an effective means of detecting illegal drug use. In the U.S., dozens of trucking companies and many Fortune 500 companies are using hair tests as a means to improve their workplaces. Their adoption of hair testing stands as a strong endorsement of it. If so many large, sophisticated companies felt that the testing process was unsound and would subject them to costly wrongful termination claims, they would simply not use it. The widespread adoption of hair testing in the private sector stands in sharp contrast to its adoption by federal agencies. It is time for this inconsistency to be addressed. Thank you.

Dr. Cook: Our next public commenter is Carl Selavka, Forensic Toxicologist and Director of Northeastern Bioscience Associates.

Mr. Selavka: Thank you very much for allowing me to speak here. As quick background, I was the Operations Officer for the Forensic Toxicology Drug Testing Laboratory at Tripler Army Medical Center from 1987 to 1991. I am a urine drug tester from way back. I am Army, so do not hate me if you are Air Force. After that, I went to National Medical Services where I managed the hair drug testing team and the criminalistics laboratory for five years. Next, I managed crime laboratories for the states of New York and Massachusetts for another 15 years or so. Surprisingly in 2009, I returned to the Air Force drug testing laboratory and found that not much had changed in drug testing in those 20 years. My most recent focus has involved service as a Report Certification Officer for a British firm involved in the family law courts in the U.K. They employed tests from seven different labs located in the U.S., U.K., and European Union, through which I have reported hair alcohol biomarkers and hair drug test results for more than 25,000 patients who fall into the family law court system in the U.K. If the DTAB wish for me to pursue U.K.-related data that you may find helpful, I am willing to at least try. They are Brits and they may not give us anything, but we will see. They also did not celebrate the 4th of July, and I still do not know why.

There may have been a misnomer this morning. From 1999 to 2001, I was the co-chair with Don Kippenberger of the Hair Testing Working Group that convened on behalf of DTAB. DTAB asked us, through the consensus peer process, to put together standards that can be turned into the consensus national guidelines that ultimately were promulgated in 2004. It was not Don alone. Don and I worked together because we were both ex-Army. Don attended Texas A & M and I was Northeastern University. He knew how to get diverse-thinking cowboys to agree, and I knew how to take good notes. I was the one that pulled together all those words that hopefully helped DTAB at the time, and I would help you again if you need me to. One unexpected outcome of the consensus-based, transparent, and fully open process that the hair Drug-Testing Working Group followed

was we rapidly enhanced the hair drug-testing quality, sensitivity, and interpretive strengths. From the collegial process, less experienced labs, as you have heard today, learned from stronger laboratories. In many cases, arguments regarding key points of the drug testing technology, rather than nice-to-have practices, ultimately created a stronger industry for all of us to take advantage of. One of the key elements was our joint recognition that the hair drug testing laboratory must know the efficiency of its recovery process for drugs from hair. Regardless of how it determines it, the lab needs to know what that number is. Ultimately, PT that accurately challenges the laboratory process will give that kind of number back to the programs that rely on hair drug testing laboratories. During this process, some labs came to understand that their hair drug tests were not within appropriate quality standards. These labs either improved their services to meet the group consensus expectations or they stopped providing those tests. We started with 10 laboratories in the room and we ended with 8; they were a different 8 than started in the 10.

I am hoping in the best interest of speed that the DTAB would take advantage of the significant efforts of the consensus professionals involved in that earlier work, especially if the Notice of Proposed Revisions to Mandatory Guidelines serves as a good starting point for your deliberations and going forward.

I was pleased to hear significant detail today on the SOHT efforts in the consensus programmatic elements that are used by hair testing laboratories worldwide. These consensus toxicology guidelines support accreditation factors when evaluating compliance with acceptable scientific practices. Since they are employed in ISO 17205-accredited laboratories, they should be used as NLCP referenced standards in this regard. Recognition and adoption, where possible, take advantage of the speed and responsiveness of hair drug testing industry experts to the changes in drugs, science, and law that lead to routine modifications of the hair analysis interpretive guidelines that are so important to all of us. The more powerful addiction information available through hair tests is now used to help address the very large alcohol problem in many medical, family law and public safety environments throughout most developed countries in the world other than the U.S. With a conservatively estimated nine percent addiction and abuse issue with alcohol in America, it seems we need to move forward on hair and other non-urine alcohol biomarkers as well.

We are all grateful for the efforts of the DTAB as you help SAMHSA maintain these flexibilities and toxicological tools that we apply. I hope the transparency of the process and connections with the many stakeholders that we have experienced with gratitude today continues as you move this process forward. Thank you.

Dr. Cook: Our next public commenter is Greer Woodruff, Senior Vice President of Safety and Security for JB Hunt Transport Incorporated.

Mr. Woodruff: Thank you. I am Greer Woodruff, Senior Vice President of Safety and Security, for JB Hunt Trucking Company of Lowell, Arkansas, which has about 16,000 employees, with about 11,500 of which are drivers. I have had the unfortunate opportunity to knock on somebody's door and tell them I am sorry that we have killed their mother, their wife, their husband, their dad. If you have ever done that, you do not want to do it again. Many times, there is nothing you can do but say I am sorry.

We were involved in a major fatal crash in February 2005 in which our driver tested positive for cocaine. In September 2005 and February 2006, we had other fatal crashes. Twice now we had a driver who had passed all the DOT-required urine tests but then tested positive for cocaine following a fatal crash. That motivated us to seek a better way to ensure that that type of accident did not happen again. We began to seek out what countermeasures existed that could help us prevent a reoccurrence.

We implemented hair testing in 2006. Before we implemented it with our drivers, all of our management team, including myself, submitted ourselves to hair testing. We will not ask our drivers to do something that our management is not willing to do. We gave them 60 days to come forward anonymously to seek help to protect their jobs. Some did come forward, and we were able to help them stop their drug abuse.

I will show you the results of what we have found. If you will look at this first chart, I will explain it. The blue line

represents the percent of our active driver population that have been subjected to hair testing. When we started this in 2006, we had about 20 percent of the population tested. Now 89 percent of our active drivers have been subjected to hair testing. We have some long-term drivers that worked for us prior to 2006 that have not been tested, and at some point, we probably will ask them to voluntarily test so that we can move that number to 100 percent. The green line represents our DOT positive random urine rate. This rate ranged from 0.9 to 1.4 percent for years and years and years. When we implemented hair testing, that number declined to where it is now, year to date this year, at 0.2 percent, which is an 89 percent reduction. It stands to reason that if you screen out lifestyle drug users through pre-employment testing, when you test your employees later they are not drug users. The red line represents our post-accident positive urine results. It ranged from 2.3 to about a 3.9 percent over a number of years. Going back further, we would be in a similar range. After we implemented hair testing, we have not had a positive DOT post-accident urine result in over five years. Is that not what we are trying to accomplish in a safety-sensitive transportation job? We are producing results. This is a game changer, and we need to quit resisting the change that can affect the safety of our highways.

Since 2006, we have tested 64,814 paired specimens. I believe this is probably the largest group of paired specimens that exists, certainly in the transportation industry. As shown in the top right box, about 95 percent of the time the result is the same whether hair or urine is tested. The positive hair only percentage is nearly five percent. It is only 0.14 percent for a driver that passes the hair test but fails the urine test. We had 3,221 instances where the driver passed the DOT-required urine test but failed the company policy hair test. If we were not testing for hair, those drivers would be driving a JB Hunt truck today. Fortunately, they are not, but they are probably driving a truck for somebody else. There is a flaw in that they can work for another company because we cannot disclose that they failed the hair test with us; we can only disclose the DOT urine results. In the additional boxes provided on the left-hand side are those that tested positive for hair only. Seventeen hundred people failed the cocaine hair test but passed the urine test. About halfway down the chart, the data indicate that 15 people tested positive for heroin. Is that a guy you want driving a truck? I do not think so. Notice that some individuals have varied combinations of drugs. Individuals oftentimes are failing more than one drug test. On the right-hand side, notice that 90 individuals passed the hair test but failed the DOT-required urine test. These data illustrate the results of hair testing applied practically in a commercial trucking operation. I think these speak to the scientifically-sound nature of hair testing. We would not be seeing these types of results if it was not sound. We would not have zero DOT positive post-accident results if this was not effective. We believe it is working and it is sound. Additionally, the cutoff levels and the confirmation testing is the same technology that is used for urine testing confirmation. The technologies are not that different when confirming the result.

On the issue of hair color or racial bias, I do not see any research that states with any certainty that hair testing is racially biased. The results have been very small sample sizes, seven or eight people, and it always concludes with it may be. It is never conclusive that it is. There are no facts that say that hair testing is racially biased. To evaluate this issue, we engaged Dr. Thomas Mieczowkski, who was part of the 1998 industry-led working group. We asked Dr. Mieczowkski to help us understand this issue of racial bias, whether it exists or not, because we have read the research and could not find anything conclusive. He analyzed our paired drug testing results for urine and hair along with the race of the individuals based on their self-designation from the applicant data record. His results from December 2009 are shown in attachment one of your handout. He broke out the urine and hair test results by black and white individuals. In the right-hand column is the ratio of black to white with one set of data for cocaine and another set for marijuana. He concluded that there is no statistical difference in the ratios of black to white positive or negative results for either cocaine or marijuana. Any differences between races are not because of the specimen type but must be related to something else.

On September 28, 2012, the United States Massachusetts District Court ruled on the civil action of Jones versus the City of Boston. This is a case that originated in 2005 when some Boston police officers alleged that there was a disparate impact because of the use of hair testing by the police department. After presentations of years of test results, statistics, scientific evidence, and expert testimony from both sides, a summary judgment was entered in favor of the Boston Police Department. Of particular interest was the judge's application of the Equal Employment Opportunity Commission (EEOC) guidance known as the four-fifths rule. This rule states that a selection rate for any race, sex, or ethnic group which is less than four-fifths or 80 percent of the rate of

the group with the highest rate will generally be regarded by the federal enforcement agencies as evidence of adverse impact, while a great than four-fifths rate will generally not be regarded by federal enforcement agencies as evidence of adverse impact. Because the passing rate for African Americans was at least 97 percent of the passing rate for Whites, the judge stated that the EEOC would not regard the results as an adverse impact. The judge thus issued a summary judgment in favor of the Boston Police Department. Following that ruling, JB Hunt reexamined the last year's worth of data immediately preceding that ruling and applied that four-fifths rule. Shown here is a year's worth of results applying the EEOC criteria that was referred to and used in the Boston case. The different ethnic groups are listed in the right-hand column. Also shown are the number of passes or fails, which would be positive and negatives; the total number of tests administered for a particular ethnic group; and the percent pass rate. All the groups are in the mid 90s or above for their passing rates on hair testing. To apply the criteria, the highest group was Whites at 98.18. 80 percent of that is 78 percent. Thus 78 percent or above is needed to not have a disparate impact. The right hand column provides that applied rule data. You would conclude it is not even close because there are no data even in the low 90s. Examining the results of Dr. Mieczowkski's analysis of ratios, and the EEOC application in the Boston case, and our EEOC application of the four-fifths rule, there is no racial bias and there is no adverse impact. We should not even be arguing about that anymore. There is plenty of evidence to suggest there is not.

I have provided a list in the handout of a number of lawsuits where drug testing has been upheld. It is used in employment, child custody, parole, and probation cases; it is used by law enforcement, court systems, and industries. There is a list of cases there, including the New York Supreme Court, the Nevada Supreme Court, the Florida District Court of Appeals, and numerous others. Thus, I would argue it is legally defensible. It is been defended in a number of cases. It is scientifically sound, it is forensically defensible, and it is not racially biased.

What about external contamination? We have heard evidence here about washing techniques. Washing techniques remove the external contamination. The method of determining the quantity in the final wash from the quantity found in the liquefied hair, followed by applied cutoff levels gives the benefit to the donor. External contamination is an issue that can be dealt with.

The incorporation of hair testing into the Mandatory Guidelines for Federal Workplace Drug Testing Programs is in the public interest. It will allow DOT to incorporate hair testing as a specimen for appropriate types of tests associated with safety-sensitive transportation workers. DOT cannot move forward until SAMHSA promulgates rules into the Mandatory Guidelines. We need you to act. Additionally, incorporating hair testing into the Mandatory Guidelines will further the intent of Congress, who outlined under Section Two, Number Three, of the Omnibus Transportation Employee Testing Act of 1991 that great efforts must be expended to eliminate the abuse of alcohol and use of illegal drugs whether on duty or off duty by those individuals who are involved in the operation of aircraft, trains, trucks, and busses. Congress expressed that great effort must be extended. Has it been? Great effort must be expended, and illegal drug use while off duty is unacceptable for those in safety-sensitive transportation jobs. Taking a position that we should not be concerned with what truck drivers do while they are off duty or that we are not concerned with lifestyle drug use is in direct conflict with the intent of Congress. Most of the drugs that safety-sensitive transportation workers are tested for are illegal and habit forming. Thus, they cannot decide to only use them while on their time off. They are likely to use them while driving a truck, flying a plane, or driving a bus.

I want to encourage you to expend the greatest effort to consider the longer detection windows afforded by hair testing that address the illegal drug use while off duty that Congress expressed concern with. Based on JB Hunt's experience over eight years with 68,000 drug test collections of paired urine and hair from the same individuals, we believe the facts support the use of hair testing and that the combination of hair and urine testing can produce the most effective outcomes.

I hope that you will begin where DTAB left off 10 years ago. The group of leading experts in the field of toxicology and drug testing concluded ten years ago that hair testing was a viable alternative. That is why SAMHSA promulgated rules that included hair testing. As you examine this issue, you will conclude the same

thing the DTAB did 10 years ago, that hair testing is a viable alternative.

I want to mention one thing on PT. Some of the labs performing PT 10 years ago met all the standards. Why would we not establish a standard that would allow those that can to do it? Because some cannot? That is like going to the Olympics as a high jumper, and I clear the opening height. The next jumper from a different nation gets up there and cannot clear the bar. So we decide not to have a high jumping competition anymore because not everybody can clear the opening height. There are labs that can meet all the standards that were set by the DTAB 10 years ago. We elected not to set a standard for others to pursue and meet because there were labs included in the process that could not do it. Now that sounds absurd to me. We need to move forward with this. I appreciate the opportunity to talk to you. Thank you.

Mr. Bonds: Janine, I have a procedural question. Whenever public industry collects data and does research for their own company, I think that information would provide some value both to us and probably the author if we could pose some of the same questions that we do to evaluate other data that we are given. I do not know if there is a possible venue where we could either. If Greer would make that data available to us and be able to follow up with our questions, we would have thorough understanding of how it was collected and how they arrived at those findings.

Dr. Cook: The procedure is that the Board does not make comment on or have discussion in the open public session on public comments. We do discuss these in the closed session. We can ask the shareholders for more information.

Our last registered public commenter joining us remotely is Brian Drew, President of Nationwide Medical Review. Brian, if you press star one, you will be able to speak. Operator?

Operator: I am not showing anyone in the queue.

Dr. Cook: Is there anyone located here onsite or remotely that would like to give public comment at this time?

Mr. Shultz: This is Ted Shultz. Most of you on the Board know who I am. For those of you who do not, I am a toxicologist and also an attorney. I have been corporate council to one of the big drug testing laboratories. I am also the president of the American Association of Medical Review Officers, for which I have done most of the training for MROs. For the last nine or so years, I served as the public director of the American Board of Forensic Toxicology, which certifies forensic toxicologists and crime laboratories. Also, I am a big proponent of hair testing.

In defense of SAMSHA, and in an open note to the industry, one of the great accomplishments of this process has been preventing this program from turning into a litigation program. In the very early days, there was concern about the nature of what we were doing when the federal government mandates a drug test, which is considered to be a search, and all of the rights and privileges that go along with that. A great deal of effort was exerted into making the laboratories bulletproof, and to a large degree, that has been successful. The evidence of that is 30 years of success with a very low rate of litigation, whether it is successful or not. There has been very little successful litigation. Much of it is because of the sort of things we are processing now - QA, QC, PT, and GC/MS. When the federal government enters into this testing, it has to be not beyond a reasonable doubt but beyond any doubt. If you can raise a doubt, you have a litigable issue. I have worked for the Army as well as a whole constellation of federal agencies. For example, military people are entitled because it is a criminal environment. With a criminal case, they are entitled to council. They will litigate it if the stakes are high. When we talk about drug testing as a product, number one it is an intangible product. You do not see it, you cannot pick it up, and you cannot turn it around and shake it. In the service industry, you do not know what you have bought until it is not delivered. Here when it is not delivered, you are on the steps of the courthouse. You do not want to be on the steps of the courthouse. You do not want to even be near the courthouse. You do not want to have any litigable issues in all of this.

From my perspective, I see three facets that we have to struggle with. Part one is what this august body can do

and has done reliably is the science. The science is the reliability, accuracy, and validity of the data that are generated. The other facet is the application. Where are we going to use it? What is the social construct? What is the legal construct for application? Is this going to be a post-accident test? Is this going to be a return-to-duty test? Does that sound like it makes any sense? We have an environment where we have high risk and low risk testing. I have been very involved with the implementation of pre-employment testing in the railroad industry. I do not think one can get a job in the railroads today without submitting to a hair test, which is done at the front end. It is done quietly, with no fuss, no muss, or no outrage that the government has not mandated it. They do it because there is a return on an investment, which is the same thing that the trucking companies have seen. They have lower recidivism, lower positivity rates, and they get a better applicant. That is all worked. What is the difference? It is a different industry in the trucking industry. The trucking industry is its own worst enemy. Why? Because there are very good legitimate companies competing with all kinds of others, shall I say. The issue has been not maintaining the quality of their drivers but the quality of their competitors' drivers, with everybody on a level playing field. That is a business issue. The point is that why I do not hear boo out of pre-employment testing is that it is low stake testing. When you start testing people who have a lot at stake, they will litigate it. My third facet is it is part of the world of forensic sciences. We have seen forensic sciences evolve over the last couple of years, trying to remove the subjectivity, by determining whether this is a forgery or not. The voodoo of forensics is being clarified. It has a way to go. We have seen this in the Zimmerman trial. Both sides had experts perform digital analysis of the yelling voice. The court said it was not scientific, so they kept everybody out. But 10 years ago, everybody would be in the courtroom to listen to the digital ups and downs to determine whose voice we were listening to. The third piece of this is the interpretation. The interpretation of the data is where we have the biggest issue. We have had that issue and dealt with it in urine testing, passive inhalation, and passive ingestion. As a matter of policy, sometimes we say passive ingestion is possible, but it will not be an alternative medical explanation. We are not willing to accept that. We have also done studies with the passive inhalation, and we have a general consensus. Back in 1985, my boss asked me what is the liability for us as a laboratory for providing this service. I said that is a pretty good question. I do not think I am smart enough to figure that out. I surveyed the toxicologists at the time and asked them to rate the defensibility of all the various methods. The results were published in the Journal of the American Medical Association, with GC/MS listed as the gold standard. Now, the gold standard has obviously evolved, but it is a chromatographic method that defines it.

Claims and interpretations, such as 100 percent certainty that drug was accidentally ingested, that the drug came from the environment, or that drug was used, are not the role of the laboratory. The science is not there yet. That is my only beef with all of this. The claims issue is where the voodoo is. That is the issue that needs to be defined. That does not mean that hair testing cannot be used because we still have some voodoo in urine and we still have some policy issues that we have to, as a matter of policy, accept. Those are my comments, and I want to thank you for this opportunity.

Dr. Cook: I will end the public comment period, as well as adjourn this meeting. Thanks to everyone for attending.

Adjournment (5:30 p.m.)