Nucleic Acid Tests
John Butler, Ph.D.

DR. TEUTSCH: Great. Thank you so much, Dr. May. We are going to take the next three presentations in a row and then get questions after that. Let me turn it over to Dr. Butler, who is going to talk to us about nucleic acid tests.

DR. BUTLER: Thank you for the opportunity to address the Committee today. You will notice the slides that you have will be different from mine. I will have a few new ones. Some of them will be hidden, so I won't show all of them, in the interest of time.

What I want to show are some of the things we have done in the past and what we are trying to do now with the new Applied Genetics Group that has been formed within the Biochemical Science Division at NIST and within the Chemical Science and Technology Laboratory, and then some of our thoughts for the future.

In terms of the past, most of our experience has come with doing forensic DNA testing, developing reference materials and methods, genotyping assays, and new technologies for improving forensic DNA testing. This is something that has been well noted in the press in terms of the need for good standards and quality measurements.

In terms of the present, two months ago, on October 1st, we formed a new Applied Genetics Group, which is, again, bringing the expertise we have with developing reference materials for forensic purposes and now applying that to clinical genetics and also agricultural biotechnology efforts, like genetically modified organism detection.

We have some done some work with genetic genealogy and DNA ancestry, trying to help with improving their nomenclature and how testing is compatible within things.

I will finish with just a few thoughts on some planned genetic testing and some of the things we would like to work with. For example, the CDC's GeTRM program. We want to collaborate with them on things.

In terms of our initial efforts and interest in getting into forensic DNA, Congress passed the DNA Identification Act in 1994, which gave the FBI authority to establish a national DNA index system, or national database for DNA testing.

As part of that, there was a DNA advisory board that was formed. One member of that was from NIST. From that came quality assurance standards which now govern how all forensic testing is done in the United States. These standards have also been adopted for testing around the world as well.

Standard 9.5 within the section on analytical testing says specifically that the laboratory shall check its DNA procedures whenever a change is made against an appropriate and available NIST standard reference material or a standard traceable to a NIST standard. This is what has driven most of our efforts in forensic DNA testing, trying to provide information that can help with the underpinnings of quality measurements for forensic laboratories.

This is a new slide here that I just added showing that at the highest level, the community level, there are quality assurance standards to make sure that there is also, of course, inter-laboratory studies to make sure that everybody can talk to each other in terms of their data.

Within the laboratory, there is the American Society of Crime Lab Directors Laboratory Accreditation Board. They have accreditation of laboratories. Audits are performed, usually annually, of laboratories to make sure that they are compliant with the specifications there.

Each individual forensic DNA analyst must perform two proficiency tests per year on any type of testing that they are doing, plus they are required to have continuing education to keep up with new technologies.

The next level is the instrument or the method level, where we have validation of analytical procedures. This is where the NIST reference materials come in. You have a traceable reference material to make sure that your instrument or your method is working properly.

Next is at the protocol level, where you have standard operating procedures to make sure that the instruments are used consistently from analyst to analyst and so on. Each data set has its own standard materials that are run, positive and negative controls, and so on. Allelic ladders are a mixture of DNA samples to show all the possible alleles that would be seen.

Individual samples have internal size standards that are run with them. Then we have interpretation of results that are confirmed by a second analyst. Finally, of course, when you go to court, you have defense attorneys and defense experts that can examine your data as part of discovery requests. That provides another check and balance on how forensic DNA results are done.

So, all the way from the community level to what is presented in court there are checks and balances with things. The reference materials that NIST provides are only a small piece of the validation of the analytical performance of something.

Over the years, there have been a number of different technologies that have been used. For each of these different technologies we try to have a NIST reference material available to help with this. The first is, of course, the restriction fragment link polymorphism, developed in the late '80s. That was the initial DNA fingerprinting or DNA typing that was developed.

Then there became polymerase chain reaction-based tests. The next series of reference materials was SRM 2391, which has been available since the mid 1990s. Then we have had ones for DNA sequencing and mitochondrial DNA and, most recently, for Y chromosome testing.

The technology in some cases is no longer used and therefore reference materials get phased out. Then there are growth areas in terms of new markers and new information that can be added to the same samples and certified on the same samples.

This is just to illustrate what we do on the genetic tests. On the top right, you see a picture of the DNA samples themselves. There are 12 different samples that are provided for this particular test. Then there is a certificate of analysis that provides genetic data for each of those samples.

In this case, they were characterized for 22 autosomal, short-10 and repeat markers that are used in forensic testing around the world. We have just recently added 26 new STR markers. It is basically a value added to the same reference material. So the DNAs haven't changed. We have just added more certified information to them.

We have also tried to encourage the slowing down of the consumption of these because they are expensive to make and certify. We tried to help laboratories make traceable materials instead of just using straight off the shelf the reference materials themselves.

These are the basic steps in forensic DNA testing. You collect the sample, you extract the DNA and quantify how much DNA is present, perform a multiplex PCR application. Then you look at the short tandem repeat markers and interpret those results, and then put those results in a database where they would be checked against the frequencies of alleles to determine how common that particular profile is. That is what would be presented in court if they match.

So the reference materials only focus on the actual typing results that are produced. There are many other aspects of the process that could have reference materials, but right now we are just focusing on the separation of the DNA itself.

We are looking at short tandem repeats. That is what is used in forensics where we have primers that target a repeat region. The number of repeats is then converted. The overall size of the PCR product is measured and then the number of repeats is what is actually considered in the final analysis and what is reported. In this case, 11 GATA repeats is what is recorded in the database for that DNA profile.

That measurement is made against an allelic ladder, which is a mixture of alleles. You can see in this case, just showing two samples, one that is a 16/17 and one that is a 15/16. Both those samples are compared against an allelic ladder that a commercial manufacturer produces. They check that allelic ladder against the NIST reference material.

There are different sites that are used throughout the human genome for forensic testing. In 1997 the FBI defined 13 core loci. There is also a sex-typing marker that is used called amylogenin that is present on X and Y. Then there is some overlap with Europe. So our reference materials are also used in Europe, though they use slightly different genetic markers for their testing there.

Now, within the U.S. we have over 6.5 million profiles on the database. A laboratory cannot put their results on the database unless they have run a NIST SRM to make sure that their results are accurate and so on.

Again, a little bit more on the STRs. We are measuring the base pair size, converting that back to a repeat number, and that is what is being stored.

This is also used for paternity testing. Our reference materials are used to help with making sure that paternity testing is done properly. The American Association of Blood Banks, AABB, is who oversees how paternity testing is done.

This is what a full DNA profile looks like, just to illustrate the process. An internal size standard is run with every sample. Then we have the individual samples compared to an allelic ladder to actually get the genotypes for each individual site. The measurement is performed by the allele size.

Another thing that is important to point out, of course, is that different genetic tests may use different PCR primers and therefore, because of binding site mutations, may produce different results because of allele dropout or null alleles. This is just to illustrate one example with a NIST SRM 2391b.

The Genomic DNA 8 actually has a dropout at this marker on chromosome 16 with a new kit that just came out from Applied Biosystems. You lose Allele 11. This becomes important as laboratories are trying to verify if their procedure is working properly. So we go through and do a lot of work to calibrate and sequence the regions and define why a particular new assay or kit doesn't work properly.

We are funded primarily by the National Institute of Justice to do this work, as well as internal NIST funds. We have reference materials, as I mentioned. We have standard information. We have conducted a lot of interlaboratory studies. On the technology side, we are constantly developing new assays and new software. We have training materials. You can go on our website, which is the STRBASE website, and download PowerPoints and other workshop information to help people learn more about this.

Just to get to where we are now, you will hear about some work going on in the Analytical Chemistry Division in just a moment. We are within the Biochemical Science Division. It is all underneath CSTL. We just, as recently as two months ago, formed an Applied Genetics Group, which is one of six groups doing work with genetic testing. These are the people that are involved there. Marcia Holden and Ross Haynes are new additions to our group, the former forensic group. We are really expanding in this area.

Our mission is to advance technology and traceability then with quality genetic measurements, continuing to help the forensic testing community but also clinical genetics, the ag bio tech, and then also DNA biometrics. There is a tremendous interest in this area and speeding up the process of DNA testing and making sure that is done accurately by the intelligence community, and so on.

This is some of our group expertise and funding sources. We have primarily, again, expertise in reference material characterization, construction of new assays, a lot of work with sequencing, SNPs, STRs, and so on. Our primary funding is coming from NIJ, but we are also getting internal funding from NIST. We plan to strengthen our portfolio in the clinical genetics area.

DR. TEUTSCH: Dr. Butler, I hate to interrupt you, but we will need to wrap this up so we give everybody a chance.

DR. BUTLER: That's fine. These are our reference materials that are available right now. There are some slides from Mark Salit here on some of the RNA work that he has been doing.

We have been trying to help with nomenclature to help the genetic genealogy community to make sure that they are getting consistent results across laboratories.

This is one of the new ones. We are working on Huntington's disease, trying to have alleles that appropriately define each of the characteristics you would expect to see with Huntington's disease.

We have to decide, and we welcome input, in terms of what types of materials should we certify. We can certify for a sequence, a specific genotype, and of course, the quantity of DNA that is present.

We want to continue making information available to the public, as we have with our forensic stuff, and make that available for clinical diagnostics as well. Feel free to contact me if you have questions, and thanks again for your attention.