Elaine Lyon:
First of all, thank you for inviting me to do this. This has been interesting for me to kind of pull some of the concepts together to talk about, and I very much have enjoyed going through this and working with you, and I really appreciate the opportunity to talk with you.

I'm going to be focusing on the ACCE framework, and my scope is going to be with molecular assays. And I spent the last 18 years trying to fit genetic testing into a clinical chemistry testing laboratory. So, ARUP is mainly chemical chemistry, microbiology, and immunology, and trying to take those concepts and apply them to molecular genetics has been challenging at times.

So, the one disclaimer I have is that these are my opinions, and that even within the field, you will get disagreement. I've been in many discussions about it. My thinking has evolved. It is still evolving. So, HHS, as we understand more. I wanted to thank Heather for working with me with the BCBSA and the policy, so I'm -- I was using that as a framework, but I'm also going to be talking a little towards the future. Even if we're not there or we may not be there quite yet.

The other thing I wanted to comment on is my background is inherited diseases, so I have a few slides on molecular oncology, but it is such a different issue that I just -- I've talked with people about bringing people on and presenting just on oncology. So, even though I have a few slides with it, I'm going to focus on molecular genetics and inherited diseases.

So, I'm -- but everybody here is aware of the ACCE framework, analytical and clinical validity, clinical utility, and then the ethical, legal, and social implications. And then, the purpose of the test, which -- actually from Fryback-Thornbury modifications is to reduce the morbidity and mortality, and provide information to the patient and family members, and to assist with reproductive decision making.

So, the frameworks -- the figure of the frameworks is coming from that paper. All of them have basically the same ideas, although there are some -- other points that I want to make is that in my mind, there's a difference between a biomarker and a mutation. So, when I hear the term biomarker, I think of something that is showing some type of an association, and it can give you a relative risk over a general population.

These would be either clinical trials or GWAS studies to show an association. They may be gene expression patterns. These are more likely to be proprietary, and I will tell you, I don't do many of these in our own genetic tests. And I would say, if you're looking at these type of tests, you'll need to talk with that -- the laboratory who has developed that because most of the time, they have that information, and they may be proprietary. So, I'm really talking about mutations, or -- in the ACMG's terminology, they've asked us to call them pathogenic variants. So, everything's variant, and then these would be pathogenic.

So, most of the testing I do -- the vast majority, we're looking for causative mutations. There's many Mendelian disorders looking in the germ line. Now, that some of them will then cross over into the oncology, looking at some of somatic variants, looking for driver mutations, for drug susceptibility or resistance.

To start with analytical validation, and just a broad definition for me is does it detect what we claim it detects? We do the accuracy, and we do precision studies, and our accuracy determines the analytical sensitivity and specificity. When we look at this, we needed to find what regions we're look at and interrogate. Are we going to look at common, targeted mutations, or are we going to sequence the entire gene? If we do sequence gene, or possibly, we may only want to target a few exons, we traditionally look at intron-exon boundaries, as well as all coding regions. And if we know of a deep intronic mutation or a regulatory mutation, we can put that in as well, but those are not evaluated all of the time. And genes have a very high rate of having deletions or duplications associated with them. Mainly [inaudible] genome. But my analytical validity is defined by which regions I interrogate. We know some of the perform characteristics and their interfering substances. Mainly, it's Heparin. And Heparin just interferes with PCR. Now, we know how to handle it, so if we get a Heparin sample in, we need to dilute the DNA, and dilute out the Heparin so it doesn't bind the magnesium in the PCR reaction. But the other ways that the performance is affected is by rare or unknown variants of the primer of probe site, and some of them may be creating secondary structures. And so, we would be getting -- possibly, an allele drop out, meaning that instead of looking at both copies of the gene, we're only looking at one copy of that region of the gene.

Or a probe sight, if there's a different mutation in what we're looking for, it may show the characteristics of that mutation. And so, there's something there, but it may not be the mutation we're looking for. And so, that is what affects our analytical validity, and those are things that we cannot completely control --

No assay will ever be 100 percent sensitive, and 100 percent specific. The other performance characteristics are if we need to look for mosaicism or low mutation levels. Now, this is very important in oncology. It's not as important in genetics, except for a few genes where we really need to look for mosaic levels. And at that point, we need to establish what our limit of detection is.

Some people use this term as also a -- the sensitivity of the assay. I kind of keep the sensitivity -- the term sensitivity, I like to keep it to referring to the accuracy, and then refer limit of detection to tell you how low of a, you know, what percent of mutation am I going to be able to detect?

For the most part, the molecular technologies that we use, they all have a very high performance level. It's -- it is more in the design of the assay than the technology itself. We all use primers, probe hybridization, sequencing -- so these are all very well established, and there's a lot of different varieties, but for the most part, they pretty much have very, very similar accuracy and analytical characteristics.

One other thing about the analytical validity is that even once we bring a test online -- so it's not in our -- so, beyond our validation of the test, once we bring it online, we have a continual evaluation through the CLIA (Clinical Laboratory Improvement Act) program and the College of American Pathologists to do proficiency testing, or some way to assess that we are continuing to get the same type of results -- the analytical performance.

I don't think anything there could be controversial, but once we start talking about clinical validity, people define this a bit differently. So, again, broadly, I look at clinical validity: does the test correctly identify affected or unaffected individuals? And my question that I've been talking with people is, is it the analyte or is it the assay that determines clinical validity? In my mind, it would be demonstrating for inherited diseases that this -- that mutations in this gene do cause this disease. And then, whether you identify them by standard sequencing or a targeted mutation, or anything else, that validity that this gene -- mutations really do cause this disease, is a way to describe clinical validity.

And how do we know that mutations in a gene cause a disease? Well, mainly because of linkage studies of large families, functional analysis, some case control that truthfully, most of them have been more of cloning the gene. At first, we could clone the gene from a known protein sequence, and then, we've been able to clone a gene by the genetics, by the family studies, as opposed to knowing the protein. So, we can identify genes even when we don't know what the protein is.

Again, the clinical validity is going to depend on what region you interrogate, as well as how you define the phenotype. And I wanted to use an example for the F8 gene. And so, F8 -- mutations in the F8 gene affect the factor VII enzyme, which then causes hemophilia A. The most common severe mutation is an inversion of a portion of the gene. And so, you can't pick up the inversion by sequencing, so testing is for that first inversion, and then, if that is negative, then you go on to do sequencing and -- or deletion analysis.

Now, if you just look at the inversion, the clinical sensitivity of that would only pick up maybe about half of the individuals with severe mutations. So, the clinical sensitivity for just the inversion test is going to be approximately 50 percent. If you do the inversion test followed by sequencing, and then followed by deletion duplication, then the clinical sensitivity for hemophilia A becomes very high. So, it's not necessarily the method that you test it with, but it is what you test. Do you test for the inversion? Do you sequence the gene? Or are you looking for deletions?

And with that said, we also needed to find this -- are you talking about -- what is the clinical sensitivity of this assay in detecting individuals affected with hemophilia A, and that's the narrow definition of what I believe should be the definition. If you expand that and say, what is the clinical sensitivity of this test to detect -- to diagnose somebody who has some type of a bleeding disorder? Well, it could be Factor IX. It could be von Willebrand. And so, therefore, the clinical sensitivity decreases if you use a broader definition of the symptoms, rather than the specific disease. So, whenever possible, I think we need to define our clinical sensitivity by the narrow disease that it is going to be looking for.

Along with this, I have heard and I've seen the use of positive predictive values and negative predictive values, and I'm not quite sure where this fits in. Is it really a measure of analytical validity, or is it a clinical validity? I would say it probably goes into the clinical validity type measure more than an analytical validity. Or does this really measure clinical utility?

Now, with single gene disorders and the mutations I'm looking for, looking for causative mutations -- truthfully, I'm not sure how to use positive predictive values and negative predictive values, being that to me, the clinical sensitivity is the -- or the clinical specificity is the stronger value. If people have a way how to use this, and I'm really open, and I'm trying to learn this, of how to use these positive and negative predictive values for single gene disorders. Now, there's other times that I do, and I understand it but one of the other questions is does it then becomes dependent on the population?

So, if I'm using my test for us to diagnose for affected individuals, it's going to have one positive predictive value or negative predictive value. If that test goes into a population screening, the vast majority are going to be negative. The specificity may be high, but the -- or the negative predictive value increase, but the positive predictive value will go down. And I look at this and say, "That's the use of the test, but not necessarily the test itself," which is why I am putting the PPV and NPV more into a clinical validity or the clinical utility category.

Now, there's a lot of complications that happen when we talk about clinical validity, and these concepts that may interfere or may -- that we need to be aware of when we're talking about this, one is penetrance or expressivity. If we have a highly penetrant allele, then if we identify it in this individual, this individual is going to have symptoms of the disease. Now, if it's a low penetrance allele, then I would see how a positive and a negative predictive value would be more applicable for low penetrance mutations. But still, most of the things we're looking for are the highly penetrant ones. The expressivity will simply tell you the range of symptoms that may be associated with this mutation, or with this gene. There is a clinical overlap between pathogenic variants and multiple genes that cause similar phenotypes. And this is where the laboratory -- clinical laboratories, and even the clinicians are really embracing the gene panels, and to look at multiple genes at the same time because it is difficult to do one gene at a time. The phenocopy is also difficult, and it depends on the disease, on the gene. But if that's -- if there's other reasons and other things that are causing similar symptoms. So, an example of this is the BRCA1 and 2mutations. If your phenotype is breast cancer, then mutations in these two genes are low-- and going to be a relative small number of individuals with breast cancer that will have mutations; however, if you it define it as a hereditary breast or ovarian cancer syndrome, then our clinical sensitivity then increases.

And I think I have this point on several different slides, so if you see it again, I apologize, and maybe it's just that I want to drive this home. For me, the same test is used for diagnostic, for predictive and carrier testing. So, they have different reasons to perform the test. The analytical performance is the same for all of them. I can test all of this, but clinical sensitivities are going to be different. Two points that I wanted to make is that there are -- again, we don't routinely look at deep intronic variants or regulatory variants because most of the time, we don't know how to interpret them anyway. So, we try to limit ourselves to the regions of the gene where we know that we can -- we have a good chance of interpreting them. This is the same thing as if they are in a gene that's not well understood, and there are studies -- there are, you know, publications coming out now saying, "This gene -- this mutation has been identified in this gene with this person who has symptoms." That is establishing clinical validity for that gene. And so, more studies really need to be done before I bring that gene on -- and testing for that gene. So, when we design a panel, we are very careful to make sure that all genes in that panel

independently have established clinical validity. One of the things that have been very challenging as we go to gene panels and looking forward is really knowing what are the right genes to put on. You know, which are -- you know, which should be included. And NIH has a fund -- has funded a project called ClinGen, and I've been on a couple of these working groups, and I really like it because they bring the experts of the field together with the laboratory experts to go through the gene, and I'm hoping -- and I guess I have a lot of hope, that perhaps, this project can come up with better guidelines for clinicians and laboratories and payers that can use, and looking to see what is the right thing to do.

So, as we go into clinical utility then -- and I have taken this as a -- the modified ACCE by Fryback and Thornberry [spelled phonetically], where they have expanded a bit the diagnostic -- diagnosis into the diagnostic thinking efficacy and -- with the idea that we do a number of testing where we're being asked to rule out -- that there is a differential diagnosis. And what we can give them back is that we -- if we didn't detect the mutation, what is the residual chance that this person is still affected? Well, it can be reduced, but we can never take it to zero.

One of the important aspects of molecular genetic testing is that at times, we can stop the diagnostic odyssey. We can stop, prevent additional testing by identifying the causative mutation, and at that point, do the appropriate follow up and the appropriate monitoring of these patients. But obviously, there's the other one, such as the therapeutic efficacy, and what is the drug response? And then, they have -- called what they say the patient outcome efficacy. So, patient management. Does this improve outcomes and all of our goals? But also, a prognosis. Does it determine aggressiveness of the disease, which could also tell you how aggressively you may want to treat? And then, with predictive, and using this as more of the pre-symptomatic individual. So, you know that there is a familial mutation, and you can identify them before a patient has symptoms, or potentially, as carrier testing in the family, et cetera.

Then, the last on this slide a societal efficacy. And I very much like thinking about this because at this point, it's the proper use of the resources. Either the resources, or often, in genetics, we're looking at community resources for individuals, such as Fragile X syndrome, or other types of inherited mental type diseases, where they do need additional schooling aids, et cetera.

And I put this slide, and I didn't take it out because I really do want to let you that I do think showing utility is important, and not just to get reimbursed. That I really feel like it's important for us -- and we work as a laboratory, to show and help clinicians in ordering the correct test. And also, how to interpret them, and letting them know what this test can and cannot do. And in doing so, I think we demonstrate the value of genomic medicine in a whole. I do -- I believe in it, and believe that it has a very strong role in our medicine. Now, it's not the end all of everything. There are a lot of other things happening other than genomic medicine, but I did want you to know that I want to show utility for reasons other than just reimbursement.

Now, the definition of clinical utility is going to be different for different people. The narrow one that I have heard is people saying that the only true clinical utility is determined – drug or dose, and you have to improve your outcomes. Now, this is going to be a very, very narrow definition that eliminates the importance of -- or minimizes the importance of a diagnosis. And for inherited diseases, a diagnosis is very important, and that there is inherent utility in

establishing the diagnosis. Because without it, you don't know that you're doing the proper treatment or the proper management. And sometimes, the diagnosis is such in genetic disease -- that there is nothing we can do about it. There is no treatment. All we can do is potentially help manage the patient. But that's an important piece of information to know, as well.

It goes beyond this for a family. If you look at a -- the patient in a family, genetics really is about family. Once we identify a mutation in an affected individual, we do go on and get additional family members that also want to be tested. And truthfully, I do the best job when there is a known pathogenic mutation in the family, and then, if the next person doesn't have that mutation, I am -- that's much stronger evidence than me actually sequencing the gene again, if there has already been a mutation that's identified.

Now, for payers, I understand that they're looking for treatment and improved outcome. For regulators, they're wanting to look at the analytical and clinical validity, and they are expanding into utility. And again, for society, the efficient use of our health care and community resources. So, can we get a definition to fit all of the above?

One of the challenges with establishing clinical utility is that traditionally, it has been randomized, prospective control studies. Or if that's not available, retrospective studies with archived samples. These have been very difficult to do in genetics, for both inherited and for cancer genetic mutations, mainly because they're rare. And because they're rare disease, we cannot find many individuals with them. Or for example, with inherited, often it's a mutation found just in that family. Or sometimes, just in that individual, if it's a de novo mutation.

Some of these studies take a long duration, and what do you with individuals in the meantime? And is it ethically valid to keep things into a study when there is enough evidence that there really needs to be put into routine clinical care. And one of the problems in our genetics studies is after all of this that the results are often inconclusive. Either because they're poorly designed that realize afterwards, or that there are insufficient numbers involved.

Now, the EGAPP has been very useful, and being able to pull together a lot of different studies and doing a Met-analysis but a common conclusion from EGAPP is that there is insufficient evidence. And so, here is one of the conclusions for the CYP 2D6 testing for adults with SSRI treatment for non-psychotic depression. And so, they say that discourages the use of this for this case until further clinical trials are completed. Unfortunately, that's sometimes taken as evidence against. And they take a narrow -- and this was used for SSRI, and then blanketing it against others and saying, "Okay, it's not useful for anything." And one of the challenges with this is that it says it's insufficient evidence, which means that it needs to be re-evaluated with continuing studies. And truthfully, I don't know how often or how much these are really being able to be re-evaluated.

There is one example of 2C19, with the Plavix or Clopidogrel that the initial studies -- at first, they looked promising. Then, they didn't look so promising. And then, we identified another common variant that was actually increasing the activity of -- which was confounding the other variants, which was an explanation why those studies in between weren't able to replicate the

initial ones. So, now, they're going back, and by testing more alleles, and when we know more about the mutations, the studies are coming back actually quite favorably.

But it becomes a circular problem, if there is -- if it's not -- if there's lower evidence, it's poorly valued, it's not reimbursed, or we can't get funding so we can't do clinical trials, so there's lower evidence. So, how do we really break this circle? I was pleased, you know, in working with the BCBSA in looking at what they -- what their parameters were, and I think we're very much similar with the testing of symptomatic individuals. So, diagnostic testing -- and in my mind, I'm looking for something that will explain the clinical symptoms. And if so, we can understand better the disease course. The prognosis then -- it will help us understand the likely disease progression, and to allow us to potentially do preventive management. And then, the therapeutic, which I would love to do -- unfortunately, in genetics, we don't have enough examples of them where we determine the most effective therapy or treatment management.

Also, with an asymptomatic individual -- so, a person who doesn't have symptoms, it can be done for predictive testing. And this is usually done for a family history because of the family history in it. Again, it's so much better for us if we had tested an affected family member first so we know what those family mutations are. And then, we do do a number of these tests for population screening to identify individuals -- identify newborns. And these do have treatments involved, and sometimes, the treatment is diet. So, if they can be controlled by diet, that is absolutely wonderful. So, the testing somatic cancer cells -- again, some of them could be for diagnostic purposes, but more often, they are for prognostic or predictive purposes to determine aggressiveness of what the disease should be and therefore the treatment, or the therapy, or resistance to therapy.

Our main difficulty is that the models for clinical studies just are not working very well for us, so the fully powered studies, they're not feasible. So, how can we use some under powered or partial data? Can we model them to provide useful information? So, can we think of different ways to gain enough evidence -- supportive or adequate evidence, by looking at chain of evidences, looking at biological relationships and pathways? Once we've identified a mutation that is important and in one type of cancer, do we need to show it in all types of cancers, or can the bar be lowered to show different specimen types that it's useful in other situations?

For inherited diseases, there's approximately 4,600 known relevant genes right now. It's -- I -- it's just overwhelming to me to say, "How do we show each disease separately?" In my mind, once that clinical validity has been established that this gene is -- mutations caused this disease, it's very hard to not offer that to individuals, when -- and try to do a clinical study for it. And there's another 20 -- you know, another 15,000 or more in the genome. Many more may be shown to be medically relevant. I'm pretty much convinced we have not identified all the medically relevant genes yet. Can we show the usefulness of these, comparing the non-molecular diagnostic pathway to what we do with the molecular pathway? And I -- that may be one of the best ways to show this -- the testing for this has utility. And then, the other possible way is the diagnostic efficacy. And again, I've commented the same assay can be used for different purposes. So, I just wanted to show briefly the utility for oncology where we were looking for driver mutations that are essential for tumor progression. We may be looking for passenger mutations not driving, but may be -- may facilitate. These are mainly for prognosis

and predictive therapy. And we did an exercise. I was on a working group with the Association for Molecular Pathology, talking about clinical utility. And I will say, many of these slides are coming from that discussion. We went through this, and these are some tier one CPT codes to say, "Okay, does this test -- is this useful in the diagnosis, or in the management? Or is it prognosis or predictive?" And as you can see, for oncology, a few of them are important for the diagnosis, but most of them are for more of management prognosis or predictive purposes.

For inherited diseases, as I said, they're so rare, it's not feasible to show utility for each one. Is there a way that we can aggregate by disease type, or potentially, by method type? And one of the things that our genetic councilor pointed out to me is that it's -- that there's still a struggle because even though they are rare, we may not have strong numbers to show this, but they do have a strong clinical validity, and identifying a mutation in this may be very important. And unfortunately, these don't have CPT -- not all of them have CPT codes with them. They're kind of lumped all together into the 84179. So, possibly, one of the things that I would like to do is take this back and see if we can maybe start looking at CPT codes that can be more transparent.

One of the points, though, is that together, these are very substantive that basically, if you look long and hard enough, pretty much everybody has something that could be medically relevant in them, or a family member. From an article in in JAMA, it says 100 percent of individuals have genetic variants that could affect drug response. So, it is pretty daunting to think that genetic variants affects this many people in the population. In other words, everyone. So, here are the same type of tier one CPT codes, mainly for the genetic tests. And if you can see, most of them are actually for the diagnosis, and that is the main purpose; however, some of them can be specifically used for that management of that patient. A good portion of them can be used for prognosis, as well. But many of them -- once we can diagnosis an affected individual in the family, we can use it for a pre-symptomatic, and do it as a predictive test.

So, I think we only put one down that it was a limited -- for hereditary hemochromatosis, and that is because we have learned that these mutations are not highly penetrant. And so, they -- in this case, they would not have a very strong, positive predictive or a negative -- actually, this would be a positive predictive value.

Here is an example that I used. We're kind of a center of excellence for hereditary hemorrhagic telangiectasia. It is still considered a rare disease, but it's not really that rare. Probably one in about 20,000 individuals. And it has telangiectasias around the mouth and the fingers, but the life threatening symptom is a cerebral or pulmonary/arterial, venous malformation. And so, if that is present, that becomes, really, one of the hallmarks of the diagnosis, and what you -- what needs to be controlled. To look for this, they need to do a brain MRI, with contrast, or a contrast echocardiogram. And some of those will need -- about 20 percent will need a follow up of a chest C.T., which then also increases radiation exposure.

And you need to do this at about every five years in affected individuals. Or in unaffected individuals, you need to do it until they're at least age 40 because it's only after 40 that you can completely rule out the disease. And these guidelines are available. So, by having the molecular test available for this family, identifying it in a clearly affected individual, and then being able to

identify the unaffected individuals so they don't need to go through this surveillance is very useful.

So, I know that they -- a lot of the discussion, and we are very enthusiastic about some of our gene panels, as well, and there was a guideline that came out from the American Society of Human Genetics that I will -- that I wanted to share with you. While they said the scope of genetic testing should be limited to single gene analysis or targeted gene panels, based on the clinical presentation of the patient. I took out that it said, "If clinically indicated," and so, there is -- they do recognize that there may be some times that you want to do an exome or even a genome. But their point, which I do agree with, is that you use the most focused assay available, as appropriate for the clinical symptoms. If it is a single gene and it meets the clinical criteria, you do the single gene.

So, for example, if a child is diagnosed with cystic fibrosis because of a -- two positive sweat chlorides, just sequence the CFTR gene, or you may want to start with a targeted gene panel; however, if there is a small panel with a few genes with overlapping phenotypes, then that can improve the diagnostic yield, especially if it's a non-classic one. So, the HHT that I just showed you, the hemorrhagic telangiectasia, there's actually two major genes and one minor gene. So, even that, and even though we do a Sanger sequencing, that is still a small gene panel because we do need to look at both those genes.

Large gene panels -- these would be looking at more common symptoms. And the exomes would be if you -- if this really looks like it's a -- has a genetic etiology. But there's really no other symptoms, or no other specifics that you can look at. One of our gene panels is a Marfan syndrome, and -- well, I'm sorry. It's -- we call it aortopathy, but overlapping with Marfan is Ehler Danlos or Loeys-Dietz syndrome, all of these have some symptoms in common. One of the things they all have in common is sudden death in a close relative. If we do our single gene assy, and if an individual has a clinical diagnosis and meets all of the criteria for Marfan's disease, then sequencing the single gene is completely appropriate. We don't have a huge positivity rate because so many times, we're getting a suspected pathogenic, or a suspect diagnosis of Marfan's.

If you look at this, it's from our positive patients. Over half are ones that have a clear clinical phenotype. Less than half, then, is a suspected diagnosis. And we're picking up variants of uncertain significance for those that they suspect a diagnosis. The question, then, is are these truly -- you know, they're uncertain that they could be, or could be maybe milder mutations showing a different phenotype, or we may not have picked up what the cause of the symptoms are yet. So, when put together the small gene panel, and it's 17 gene, and each one of them has a separate clinical validity, we were able to pretty much double our detection rate, and -- of picking up the mutations.

Just looking towards the future, the exome -- if you're -- one measure is the diagnostic yield, and overall, it's at 25 percent, which isn't bad, considering nobody else knows what is going on with these individuals. If you're only looking at severe intellectual disability, it drops to 16 percent, so it's not very effective for that criteria; however, if there are other neurological symptoms involved as well, we're getting a very high -- 64 percent, which is quite remarkable.

So, just in conclusion, the current model may not be able to do everything for us. So, obviously, we do want randomized control studies, or retrospective studies when necessary. But we need to adapt some of these clinical trials to be more specific for what we're looking at. We could potentially evaluate diagnostic yield, use observational data, our linkage analysis, to make sure the gene has validity, as well as there are some functional studies that -- if we can show what these mutations are actually doing. But we also need to do understand the biological relationships in the pathways because we may not have direct evidence. And I would propose that we look at the current care versus a molecular diagnostic model to maybe evaluate the utility of it.

And then, one of the other things that I would very strongly promote is more professional input of reviewing the data, reviewing the information, so that we could better practice guidelines from professional societies. I want to thank AMP's committees that I have worked with, as well as our own internal genetics/genomics group. Thank you.

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