X-ALD 101: Clinical Aspects and Screening Methods

April 20, 2016

Transcript

Laura Russell: Hi everyone. This is Laura Russell from APHL. We're going to go ahead and get started. I'd like to welcome you all to this webinar on X-ALD 101: Clinical Aspects and Screening Methods. This webinar is cosponsored by the APHL Quality Assurance and Quality Control Subcommittee and the CDC Newborn Screening Quality Assurance Program. Before we get started, I just want to acknowledge our funding source. This webinar was supported by the CDC and its contents do not necessarily represent the official views of CDC or the Department of Health and Human Services.

Patricia Hunt: Good afternoon everyone. This is Patricia Hunt. I'm a member and the current co-chair of the APHL Newborn Screening Quality Assurance and Qualit Control Subcommittee of the Newborn Screening and Genetics and Public Health Committee. I am the Manager of the Newborn Screening Metabolic Lab in the great State of Texas. On behalf of the QA/QC Subcommittee, I would like to welcome everyone to today's webinar entitled, "X-Linked Adrenoleukodystrophy 101: Clinical Aspects and Screening Methods."

Today, we will have four presentations from experts in the ALD field. The first presentation will be an overview of X-Linked adrenoleukodystrophy, the clinical manifestations, and the treatment by Dr. Gerald V. Raymond. Dr. Raymond is the Director of Pediatric Neurology in the Department of Neurology at the University of Minnesota. The second presentation will be an overview of available screening methods presented by Dr. Joseph Orsini. Dr. Joseph Orsini is a research scientist with the New York State Department of Health at Wadsworth Center and is the current co-chair of the APHL QA/QC Subcommittee.

The third presentation will focus on follow-up and diagnostic evaluation processes and is presented by Beth Vogel. Ms. Vogel is the Genetic Counselor with the New York State Department of Health at Wadsworth Center. The final presenter is Dr. Christopher Haynes and he will discuss the CDC quality assurance materials. Dr. Haynes is the coordinator of X-ALD QC and PT Program at the Centers for Disease Control and Prevention in the Newborn Screening and Molecular Biology Branch.

During the presentations, all participant lines will be muted. There will be time after the final presentation for questions and answers. During the presentation, you can submit your questions electronically if you would like. After the final

presentation, the QA session will begin. During this time, you can submit your questions electronically as well, or you can unmute your phone and submit your questions.

We'll now begin with our first presenter, Dr. Raymond.

Dr. Raymond: Hello, good afternoon. I'd like to thank the organizers for allowing me to speak today on adrenoleukodystrophy, and the clinical manifestations, and treatment. Can I have the next slide?

When you look at ALD, it falls into a broad group of disorders called peroxisomal disorders. These are peroxisomes down here in the little figure in the corner. Peroxisomal disorders can essentially be broken down into two groups, assembly disorders of which Zellweger Syndrome, the adrenoleukodystrophy, and infantile Refsum are one category, as well as Rhizomelic Chondrodysplasia Punctata. Over here, the single peroxisomal protein disorders which x-linked adrenoleukodystrophy is the most common.

Can I have the next slide? Adrenoleukodystrophy is an x-linked disorder. It's located in the Xq2A. It has an incidence of about 1 in 17,000. All races are affected. There is no specific group who is more affected by ALD than another. It's due to alterations in the gene ABCD1 which encodes a peroxisomal ATP-binding cassette protein which then leads to defects of peroxisomal beta oxidation with the accumulation of unusual compounds called very long chain fatty acids or VLCFA. While this accumulate nearly all the tissues in the body, they affect predominantly the nervous systems, specifically the myelin, the adrenal cortex, as well as the Leydig cells of the testes.

Next slide. The original fatty acid in that abnormality was first pointed out by Igarashi et al who were examining the fatty acid component of affected brains of individuals with leukodystrophies. The impactful story is that everyone knows that the fatty acids above C18 were not present. What Igarashi did was he left the machine going too long. When he came back, he found all of these unusual fatty acids including compounds now with carbon lengths of 26, 28, 30. Now, long saturated fatty acids. The next bit was that Ann and Hugo Moser developed a plasma assay which now allowed this detection of ALD in plasma, not just in brain biopsies.

Next slide. It wasn't actually about 10 to 15 years later, the gene was finally identified as ABCD1 which is approximately 20 kb long and consists of 10 exons. We have presently over a thousand identified pathogenic mutations and about half of these are private mutations.

Next slide. The role of ABCD1 has taken some time to figure out. If you look in the peroxisome, it appears to be a transporter, and it probably transports long chain fatty acids into the peroxisome for beta oxidation. When there's a defect in the ATP as seen here by the Red Cross, the long chain fatty acids continue to go through processes of elongation. Whereas we used to wonder if it was too little

breakdown or too much synthesis, we now know the answer is actually a combination of both things.

Next slide. One of the most fascinating things about ALD is that it can present with several different phenotypes. The most common phenotype is the cerebral presentation which occurs at 35 to 40% of individuals. This is a diffused inflammatory demyelination with rapid progression and the childhood form is the most common presentation. We'll go through that a little bit more. There's also something called the adrenomyeloneuropathy or AMN which occurs approximately 40 to 45% of cross-sectional analysis of individual with ALD. This is predominantly a distal axonopathy mainly in the spinal cord.

There is also adrenal insufficiency or Addison's disease occurring in 20 to 30% at the onset although we feel that most of those individuals are going to go on to develop AMN later in life. There's also an asymptomatic cohort which I will talk a little bit more about, as well as women who are asymptomatic carriers who appear to develop an AMN phenotype in adult years.

Next slide. Childhood cerebral ALD is what most individuals think of if they think of ALD at all. This is boys who have initially normal development seen in this picture up in the top, usually with onset between 4 to 10 years of age, with the peak incidence of about 7 years of age. The initial presentations is often subtle. It could often look like ADHD or other worrying disabilities, other neuropsychiatric issues. It can often times go through a little bit of a diagnostic odyssey before it's recognized for what it is. However, once it gets going, it really does progress rapidly.

This boy over here in the corner presented at age 5 with a seizure, and as one can see from the picture, he's awake. In the lower picture, he's being held by his older brother but he is now in a vegetative state. He's blind, he's deaf, he's got spastic quadriplegia. He's passed by G2. The other important point here is that his older brother also has the same biochemical and genetic defect that the affected individual does. The presence of the biochemical and genetic defect is not particular which phenotype you're going to present with.

Next slide. The MRI is often the initial clue to the diagnosis. When you get an MRI, the image to the center here has normal myelin as dark. That bright white signal is actually the abnormality. When you get contrast enhancement, you see a ring of enhancement and almost a garland pattern. About 85% of this will present bilaterally and symmetrically in the posterior aspects of the brain, whereas, a remainder typically present frontally. It's important to highlight that the MRI abnormality precedes the clinical findings which we just discussed.

Next slide. What you see here on the top picture is a very small lesion in the back of the brain called the splenium or the corpus callosum. This is a typical sequence that we, as neurologists, look at. Then, what you see one year later is that this white band is now spread across the splenium of the corpus callosum and shows

contrast enhancement giving one a window of opportunity if one knows that this is going to occur to intervene.

Next slide. Adrenomyeloneuropathy is the other major neurologic presentation. These are young men, usually in their 20s and 30s who developed spastic paralysis, some sensory involved where they can't feel their toes as well as they used to and bladder dysfunction. It has a more gradual progression than the childhood cerebral form that we just talked about and can occur over decades. It's consistent with a normal life span but cerebral disease occurs in about 20 to 40% of affected individuals.

Next slide. What one sees on MRIs, it's very nonspecific. We just see some atrophy at the spinal cord and what one sees on pathology is just loss of myelinated axons and without that inflammation that we talked about in childhood cerebral disease.

Next slide, Addison's disease or adrenal insufficiency is the other major manifestation outside of the neurologic presentation. It's a primary adrenal cortical dysfunction. It can present acute adrenal chronically. It often times presents with hypoglycemia, difficulty finding infections, dehydration, and there is hyperpigmentation because there's an elevation in the signal from the pituitary, the ACTH, and that goes on to stimulate the melanocytes. It's rare that we see the low sodium and high potassium that is often seen in other forms of adrenal insufficiency. It's a leading cause of adrenal insufficiency in males. As I've already said, majority, we feel will go on to develop some neurologic manifestations but it may not be for decades later.

Next slide. This is a slide that indicates the onset of adrenal insufficiency in the asymptomatic population. The dotted line is set at 70 picograms per mL is the normal cutoff for ACTH. As one can see, when we look at this population, even on a young age, some less than 2, some less than 1, some of these individuals already have biochemical evidence of elevations in their ACTH which suggests that they were on the track to develop adrenal insufficiency.

Next slide. The asymptomatic phenotype were defined as boys with normal brain MRIs and individuals without adrenal insufficiency. They were predominantly diagnosed by plasma very long chain fatty acids through screening of relatives with known ALD patients. Clearly, this wasn't identifiable in the past when you actually have to be symptomatic for the disease to come to attention but as we learned, it's actually one of the more frequent phenotypes we run into.

Next slide. Also to be emphasized is the 20 to 50% of women who are carriers will develop symptoms usually in adulthood. It's predominantly a myelopathy or spinal cord disease, and peripheral neuropathy. That myelopathy manifests the spastic paraparesis dysphagia which means the prickling sensation in their feet and bladder involves symptoms which can be quite incapacitating. It seems to be a function of age. Women may not present any problems at age 20 but by 40, 50,

60, there is an increased in incidence of symptoms. It's rare that we see cerebral or adrenal disease.

Next slide. This slide just, once again, emphasizes that neither the gene defect nor the biochemical abnormality predicts the phenotype. A genetic modifier has been postulated but has not been demonstrated despite extensive genetic studies. The pedigree on the left shows what individual with that cerebral ALD and another individual in the same family has a brother who developed AMN. We have over half of our families manifest like this.

Next slide. The diagnosis, just once again, is based upon the elevation in the very long chain fatty acids, specifically the C26:0 is what most centers use. These elevations can be seen in plasma or serum. They can also be measured in fibroblasts. If you can measure them in fibroblasts, you can see them in amniocytes, and we can do prenatal testing of the tissues. From time to time, it has been used. We also know that known heterozygous women who are carriers have 20% false negative rate using just very long chain fatty acids. We do have DNA diagnosis available to us.

Next slide. I am going to speak briefly on some of the management and some of the treatment, and Beth will cover some of this information also later on. The management of asymptomatic individuals looks to predominantly genetic counseling with a confirmation of the diagnosis and screening of other potentially affected individuals. They have a younger brother, they have an older cousin, for instance. We also recommend screening for adrenal insufficiency. Then, monitoring for early cerebral disease. Essentially, it's an MRI of the brain every six months.

We often times will start doing these MRIs at approximately 12 months of age and carry this through the period of time when we think it's the peak instance of childhood cerebral disease through about 10 to 13 years of age. Afterwards, we still do the yearly MRIs after this period of time because of the risk of developing cerebral disease even in adulthood.

Next slide. The current ALD therapies are adrenal hormone replacement in those individuals with adrenal insufficiency and this really should not be overlooked. It can be lifesaving and once you do this, it's is lifelong. That needs to be very clearly emphasized. The additional aspect is because the adrenal glands have now failed, they're not able to make increased dosing at the time of stress. Pharmacologically, we need to do that. We need to give them stress dosing at times of illnesses and surgeries.

We're going to talk briefly about hematopoietic stem cell transplant or bone marrow transplant which is useful in early cerebral disease. The preventative therapy with Lorenzo's Oil is an investigational treatment. There's not enough time in this webinar to discuss all the evidence one way or the other but at this point, it is a research therapy. It is not one of the established therapies. There are also ongoing gene therapy trials and there'll be more evidence for them

going forward but they are presently ongoing and once again, we are not going to discuss them further today.

Next slide. Hematopoietic stem cell transplant, also more easily said as bone marrow transplantation is effective in the early cerebral disease. We in arrest the progression through a certain mechanism; however, in of itself, the procedure carries significant mortality, at least 10 to 15%. I have transplant-related mortality at 14%. However, when we look at individuals who have milder disease, that's what the graph on the right is looking at who have a performance IQ greater than 80, and then MRI score of less than 9, sometimes referred to as the less score, those individuals have a 92% survival rate.

This was compared to non-transplanted individuals who seem to come from the same population who at 5 years out had a survival rate of only 54%. Doing the transplant clearly means that you will stabilize disease. Given the mortality though and the morbidity of the procedure, it's not indicated in asymptomatic individuals because if they're not going to go on to develop childhood cerebral disease, you would be placing them at risk, and potentially shortening their life when they have many more years to live.

What we have determined is that the best candidates are individuals who are routinely screened by MRI. What we are looking for is that early indication of MRI lesion and being certain that we intervene with an MRI lesion is still at a manageable state. That's why we continue to recommend MRIs starting about one year of age and preceding every six months.

I think that's my last slide. Next slide, I just wish to acknowledge my colleagues and certainly, the work we have done has been a collaborative work, and I will be on the call at the end to hopefully answer any questions. Thank you.

Patricia Hunt: Thank you, Dr. Raymond. We will now hear from Dr. Joseph Orsini.

Dr. Orsini: Go ahead to the next slide please. Dr. Raymond went over a lot of the condition information. I just wanted to emphasize [inaudible 00:22:30] as far as the thing that will be important when thinking about this in the screen. As he mentioned there, three major forms, this is X-linked, and mutations in the gene do not help with phenotypes, neither genotype nor phenotype correlation in screenings. That makes things a little difficult.

The major issue is accumulation of very long chain fatty acids which have signified as C26:0. We do not screen for that compound. We're actually screening for another compound and we'll go over that shortly. It causes damage in the myelin sheath to the adrenal gland. The frequency is 117 to 120,000. We're expecting somewhere between 12 and 15 cases annually in New York, just to keep that in mind. When we go over the data, you'll see where we stand with that.

If you'd go to the next slide please. The general assay approach for screening is slightly different than the diagnostic lab. As I stated, C26:0 of the very long chain fatty acid is the marker used in diagnostic testing and that's just going to be done with plasma or serum type samples. The screen for dry blood spot, you actually look for lysophosphatidylcholine marker and the molecules are shown down on the bottom of the page.

I am not going to be going into the details of negative ion and positive ion mode testing here but the compound that you're looking at in each of those slides is what we're actually looking for, phosphatidylcholine head, which is at the end of the molecule. It's what differentiates this from the diagnostic screening. The reason for that is that the very long chain fatty acids are found elevated in dried blood spots to the point where there's some overlap with individuals with disease where their phosphatidylcholine molecules are only elevated in individuals that have disease. At least, that's the idea anyway.

Next slide please. Here's where we're going to go over some of the available options. I am sure there are others that people could come up with but this is to give people ideas of the multiple ways of which you can approach this screening. The first is to use HPLC tandem mass spec. By that, when I mention HPLC, I am indicating column chromatography mass spec.

The reason you need chromatography or the chromatography is helpful is when you look at the molecule, the lysophosphatidylcholine molecule in the dried blood spot, there is interfering compounds that no one knows the actual structure for what they are but they overlap in the MRMs on the mass spec test. The accuracy to determine the concentration of lysophosphatidylcholine is not there. You actually have to use HPLC to separate the compounds. At least, that's the idea. This is first record by Hubbard et al. I can provide any references if people want the full references but in this case, if you're going to run, people are typically using a 7-minute chromatography method to separate the compounds and you use positive ion to detect the C26:0 LPC compound.

The second approach that is available and published is to use low injection mass spec, and then follow that with second tier screening, HPLC screen. In this case, if you have a lot high volume of samples and you're able to use a 2-minute run time as opposed to a 7-minute run time. With this method, it's screened many samples. Then, go to a second tier that looks for a reduced number of samples that you would be running via the longer 7-minute HPLC assay.

The third approach which is something that's coming out in the kit form in the future is using neobase tube. Perkin Elmer has been working on it as our understanding. This is for those individuals who are currently running underivatized amino acid acylcarnitines. They'd be able to actually just turn the MRM channel on for the lysophosphatidylcholine and it would be incorporated right into the amino acid acylcarnitine test. This would be very promising for people that are currently running an underivatized method. It will not work on

the derivatized method because the derivatization procedure breaks down the lysophosphatidylcholine molecule.

Next slide please. A fourth approach is to use HPLC mass spec in a negative ion mode and this is something published by Victor De Jesus and Chris Haynes. Chris Haynes is on the call and would be talking about controls later but this method is also now being used in Connecticut, and it's a little bit faster chromatography, 2 to 3-minute chromatography method, and uses the negative ion mode chromatography. Really, I'll show you some data shortly with how well that method correlates with using the second tier HPLC in positive ion mode but that will be in a couple slides.

The fifth method that I've got that's out and published is to use flow injections. It's actually not published and is to be published. It's first reported by Dietrich Matern from Mayo and this is the approach we're taking. It's to combine flow injection mass spec test to run for ALD with the lysosomal storage disorder screening. In New York, we chose this approach because we already had instrumentation dedicated to running the LSDs for Krabbe testing and Pompe testing. To add ALD was basically take a parallel extract, and combine, and run on the mass spec.

This is an approach that could be taken by states that are planning to run lysosomal storage disorders and ALD, and they'd be able to do both on the same instrumentation, and it works really well. There are some tricks and we'll go over some of the complications with this testing that are general issues with running the test in positive ion mode primarily.

The next slide please. This is just a brief overview of the way we have set up the method. It is the modification and the Mayo method. You take a 300 millimeter or 3 millimeter punch and add 200 microliters of methanol with this deuterated lysophosphatidylcholine internal standard, extract for an hour. We removed a very small aliquot combined with LSD extract. We're taking 25 microliters of that 200. There's lots of material left.

If we're doing this, we can send material for second tier testing or have material left for second tier testing. Sample analysis is about 1 minute per sample. I've got to stress this. We have fairly sensitive mass specs and I think being able to reduce the volume and run at quicker times has been very advantageous with this assay. Then, we follow the screening algorithm which I'm going to show in a couple of slides but I want to go over some of the data that we've got to get us to the algorithm.

Next slide please. This slide shows after running the ALD assay in our early times, we were able to go back to archived specimens that were from known patients with ALD and/or Zellweger carriers. The top of the table shows what we call our ALD 1 through 10 accession numbers. The condition of the individual and then, the actual concentration of the C26:0 marker that we measured on the dried blood spot.

You can see in the accession numbers, they go from 2002 at the bottom and 2013. We implemented screening in December of 2013 and we're able to pull these specimens from our archives like I mentioned, and run them with age controls. We've found that the marker was very stable and that the concentrations are more normal or what we see in current specimens if you go back to any of these different age groups.

Where we had a little bit of a wrinkle though, if you look at it, we were also provided some samples from Mayo Group and there were three samples. There was one particular one at 0.48. You see that's the second one from the bottom with the concentration of 0.48. This is all first year measurement, first year positive ion measurement. We set our cutoff at 0.4 because we wanted to be sure we would catch that or detect that 0.48.

It's important to note though here that that child, that sample was actually taken on the child at 7.8 years of age; whereas the other samples that we ran up above were from newborn periods. We felt really confident with our 0.4 cutoff in hoping that maybe there was an age relationship to the sample concentrations in this older kid but we have not been able to actually prove that in any time-dependent studies.

The next slide please. Here's some population statistics just to give you an idea of how we do this. When we first got started testing within the first couple of months with testing and piloting, we had an average concentration of lysophosphatidylcholine at 0.22 with a median of 0.21. In a very, actually, quite tight standard deviation of 0.06 figure, this assay, when you look at group samples, one of the things I do every day is we run about a thousand a day, we'll align up the sample concentrations. Per instrument, you see the concentrations vary very little from sample to sample. You'll see a fair number of samples with results above the 0.17 but we don't see very many above our cutoff of 0.4. About 1 to 1.5 per plate of samples tested will be above our cutoff.

If you look at the ALD, the numbers below, what I'm doing is just giving you counts in our lab in our hands, the number of samples you might expect to be below 0.35, below 0.4, 0.5, and 0.6 and so on. You could use this slide to maybe estimate rates that you'd see if the test were to work identically in your lab which may or may not be the case. It would be remained to be seen.

To the next slide please. Based on the data with the positive controls, we developed this algorithm, I think, for most people may know on this call, we also have a third tier test which is molecular testing. Beth will be going more into that, but I'll go into it a little bit later in this talk. The first pass through is to look for anything above 0.4 micromolar. Like I said, that's 1 to 2 samples per plate that we'll see that's elevated that go on for second tier testing.

You can look that we do have a presumptive positive category which means that anything between 0.24 and 0.39 by our test, we'll request a repeat for that

specimen. If it comes back and it's still in that range or higher, then those individuals would be referred. If the result is greater than 0.4 or greater than our cutoff, we do perform molecular or DNA testing where we're sequencing the ABCD1 gene but it is for informational purposes only. Individuals that have a second tier test above 0.4 will be referred for follow up regardless of the molecular testing results.

Next slide please. From technical challenges, I'd like to warn people in this. There can be reproducibility issues, and all of these issues are related to the fact that there's an interfering compound that's an unknown in the presence of [inaudible]That you're trying to quantify with using an internal standard. In the presence of that interference, the interference is actually at a cost of aspartic concentration and the measured concentration is actually higher than that of the target compound we're looking for.

Ionization suppression is a major factor in affecting accuracy of the results which means you have to control for that in this test. Anybody that would want to know more about that can feel free to give me a call and I can explain more how we deal with that, but for some of the things we observed early on were age effects. If you're using aluminum foil to cover your plates, we would see the edges of the plates have elevated markers. We had to come up with a type of foil that would seal the plates better. We purchased now a heat sealing foil that Chris Haynes had recommended. This works to really control evaporation because evaporation leads to increases in the interfering compound. As far as the ionization suppression effect, it's magnified as you castrate this sample.

Some of the other things that I am not going to go over but it did affect the linearity of GALC and originally, when we added the ALD test to our LSD, we lost our signal for GALC. One of the tricks you have to do is actually put a little bit of delay [inaudible]

Dr. Orsini: You have to have a delay in between your MRMs for each of the channels you're looking at. As you go from the LSDs to the ALD channels, you need to put a delay in to allow for the instrument to come to equilibrium. Primary reason for that is the ALD marker requires more energy to make a positive state.

Next slide please. Some of the technical issues are really related to the need for the internal standard not actually matching this interfering compound. Ionization suppression is really the reason or the explanation we've come up with. When people run electrospray mass spec, the whole reason it works is that you have an internal standard that is matched to the compound of interest. In our case, they were matched to a compound of interest that's normally present in low concentrations relative to the interference. As the concentration becomes elevated for that, as it would in a case of ALD, then the results become more accurate because now, you're internal standard actually is an identical match for the compound that is present in the highest concentrations.

If we go to the next slide, it will show some more on this. This slide demonstrates ionization suppression. It was a slide I found on Google, but you can see all the points or the standards are circles and there are some blanks that are triangles. Then, you see unknowns. Those are the squares down at the lower intensities. What this is doing is basically just plotting these intensity on a per sample basis that controls in regular samples. You can see the drop off is pretty dramatic. Factors of 2 to 10, and the force down to 4000, 2000. This is something we observed too. Our blanks are very elevated with internal standard counts that you measure from the instrument. Then, the samples would be quite a bit lower.

Go to the next slide. This slide demonstrates the issue. The best way to think about it is let's just presume that the signal, if you look at the left side of the graph or plot that I have or that I've tried to show, if you had interference and target compounds producing the same amount of signal, and now you add ionization suppression because if those materials are coming from blood, there's other materials in the blood that are going to cause this suppression or signal.

What we observed is that as there's more ionization suppression going on, we actually read higher concentrations of the compound we're looking for. The instrument might read 0.2 when you have a dilution of 25 microliters of solution but if you put 50 microliters, it might read 0.3 or 0.4. Our idea or thought is that this interference doesn't suppress. The signal of it isn't suppressed as much as it is for the internal standard in target compounds.

That's probably the biggest thing we found out in how to [inaudible] I think, it explains a lot of the issues we had is you have to really control for keeping the volume you use for ALD, and the amount of solution you use from an ALD really consistent sample to a sample relative to the whole volume of the sample you're measuring. That suppression effect does cause issues and is something that can be tackled, but you have to be aware of it. What it ends up doing is you'll end up with more positives going to second tier testing but what you'll find is when you go to second tier testing, you may have had a very elevated marker at 0.6 in your first tier and it goes down to below the normal level in second tier. That's how dramatic it can be.

Go to the next slide please. This is actually an example. It's just showing chromatography. If you look at the slides, on the right side, you see a table with first tier results. These are all results above 0.4 where we moved the samples in the second tier testing. Then, you see in the second tier test results that are typically 0.08, 0.1, and that's the normal. A normal concentration for this marker in second tier test is 0.07. That's the actual real value of lysophosphatidylcholine in the dried blood spot or the real concentration with assumptions of blood volume from 3 millimeter punch being taken into consideration.

You can see that there are a couple of samples. One sample was 1.02 down towards the bottom and a red 0.87 on the second tier. First tier and second tier numbers are fairly matched as you go up in concentration but if you read a 0.04

or 0.4, 0.5, they can very often be well below the cutoff when you go to second tier. That's just demonstrating the value of HPLC method.

Next slide please. This little slide I wanted to show because I think the original papers that were out were all using second tier positive ion mode to detect lysophosphatidylcholine as an accurate way. This particular assay comparison in showing negative ion mode from test results from the Connecticut Laboratory. What we did is we compared Connecticut results from New York results for second tier, and you can see a very good correlation to the point where I think people may want to consider running a second tier negative ion test as being equivalent for using the positive ion with longer chromatography.

Because the run time is quite a bit shorter, 2 to 3 minutes as opposed to 7, this could be something that for a small volume laboratory, and that's what Connecticut shows where they're running a plate or two a day, you can just tie up an instrument for that day and make the complication of first tier and second tier go away, and basically just have a first tier assay that the run time is fairly equivalent to that you're used to with other tandem mass spec testing. It's important to note though it may be possible to reduce the run times and the positive ion. The gentleman who runs this test, Mark Barresi, has been able to lower it. We haven't proven how well that works but it's possible.

The next slide please. Third tier DNA testing, I don't want to go on to this too much. Beth is going to talk about it but we are sequencing the gene. Like I said earlier, it's not intended to reduce referrals.

The next slide please. Some data just to give you an idea of the numbers that we're seeing for those of you who are faced with screening or going to be screening for this, we've screened 531,000 infants since we started screening in December of 2013. A little more number of males and number of females, slightly more males.

Go to the next slide please. Out of that 500,000 now screened, we've had a total of 47 referrals. One of the things that's really note worthy, I think that's a very low screen positive rate. Of those 47, 18 boys were found to have mutations in the ABCD1 gene, 20 girls. We have a carrier boy which is a little hard to explain. It's a Klinefelter Syndrome which is a double XY chromosome for the boy. It has some 6 cases of Zellweger and an Aicardi-Goutieres Syndrome case.

It's important to note that that's not a disease that we would have expected to pick up with this test. If there's a false positive in the bunch, this is the one that seems to be a potential false positive. We don't have an explanation for why marker was elevated in the screen but it was also elevated in the diagnostic follow up testing. Then, there was an expired child who likely had a peroxisomal biogenesis disorder.

It's important for me to note that all of these cases, the 18 boys, 20 girls, the carrier boy, when they went on for follow up diagnosis that all of them, the very

long chain fatty acid was also elevated in the serum or serum samples that were tested. This test might be one of the best tests as far as once you go to second tier testing. It really illuminates the false positives and what you're left with are individuals with elevated marker. If they have mutations, then they're likely ALD cases but we'll go more into that.

Go to the next slide please. More data. This just quickly, in the time of ABCD1 screening, we've found 20 mutations thus far. The numbers below don't add up to 20 but we found 20. Some of them multiple times. One is indicated in the middle of this slide were found more than once, except for the new one down at the bottom of pR40, I added that. It was a relatively new mutation found. Then, we've also found 14 novels, 2 novel ones in a single person. That's where it does get tricky. Because there are genotype-phenotype correlations, it's already tricky but there's also a high number of variances of unknown significance in this particular disease. That was known prior to screening.

Next slide please. Some other findings of interest, there was a boy that was going to have to undergo surgery who has spared actually adrenal crisis because the providers knew he had ALD based on screening. They did some testing and did determine that he could have had an adrenal crisis and weren't prepared for that. Identifying the newborn, we found brothers that would be potentially affected, sisters with a basis to identify other family members as well. This is all expected when you're doing X-linked screening.

For those individuals with very elevated lysophosphatidylcholine, one of the first things we do, we look and see are they a NICU or not? If a child is a NICU and they have elevated marker, all of those cases, as far as I remember, none of them would have a mutation in their genes. Those individuals had some other peroxisomal storage disorders and they ended up being the Zellweger cases where the child that had died.

We actually do not wait for the ABCD1 genotype testing to be complete if we see that in individuals in the NICU and has elevated marker based after a second tier testing. We'll go ahead and call that unit and give them a heads up because it seems to be always the case that they've got something that is a proxesomal storage disorder in this situation. As I think I said earlier, second tier testing, the marker is elevated and the dried blood spot has also been found to be elevated in the diagnostic test.

Next slide please. We're getting close to the end. Some numbers are referral rates, 1 in 11,000, roughly 0.09%. The overall incidence is just under 14,000 incidence. In males, 1 in 15,000. The PGD incidence is 1 in 75,000. These numbers, if you recall, I think earlier, I had stated and Gerry stated to 1 in 17,000 was the expected incidence for this disease. Our incidence, it's estimated to be a little higher. I think the most important thing, and I think it's obvious is long term follow up is the only way to really end up knowing the true incidence because we do make an assumption that anybody with all of the boys and all the girls with

mutation and elevated marker that they develop disease at some point and we just don't know whether that will hold true or not.

Next slide please. My list of acknowledgements, those people in the lab, molecular lab, and people who have assisted us in bringing that screening. That's the end of my talk. We'll be saving questions for later.

Patricia Hunt: Thank you, Joe. We will now hear from Beth Vogel. Thank you.

Beth Vogel: Great, thank you. Good afternoon everyone and I am delighted to go over some information in follow up for X-Linked Adrenoleukodystrophy in New York State.

Next slide please. I'll talk about some potential outcomes from a positive screen which I think you heard a little bit from Joe. Go over the diagnostic algorithm from surveillance considerations for treatment, to counseling considerations, long term follow up, and have some educational materials.

Next slide please. Joe just went over this in detail. Just to bring us all back on the same page, in New York State, we screen for yield ALDs in a three-tier method. The first and second tier both look at C26:0 and the third tier is sequencing of the ABCD1 gene. As I go through diagnostic algorithm, keep in mind that there's a different laboratory protocol. It may change where you begin your diagnostic process.

Next slide please. From a positive screen. There's three different potential outcomes. You could have a boy with x-linked adrenoleukodystrophy, a carrier of x-linked adrenoleukodystrophy, or another peroxisomal disorder besides x-linked adrenoleukodystrophy that's also identified by the screening test.

Next slide. If a baby has a positive screen for adrenoleukodystrophy, the follow up staff in New York State will notify the pediatrician, the hospital of birth, as well as the specialty care center. The baby is brought in for diagnostic evaluation. The initial visits is with either genetics or neurology. There are nine metabolic specialty tier centers across New York State. For one of our centers, it works better to have a neurology performing the initial consultation for ALD referrals. Certainly, we encourage our centers to do what works best for them. The diagnostic algorithm is followed by the centers and additional management recommendations are made.

Next slide. The diagnostic algorithm was developed by a group of metabolic specialists from the specialty care centers across the state, as well as Dr. Raymond working with us on this. Our goal was to answer the question, "Does this baby have ALD?" and to recommend a minimum lab work and evaluation if necessary to answer that question.

Next slide. In the interest of time, I am not going to go through every little box on this piece of paper. My goal is for you to start at the very top and look at where it says positive newborn screen tier one and two. Just to orient you, the starting

point is a baby who has had a positive first and second tier. Then, for the diagnostic algorithm, you would break into two separate groups, the babies who have a mutation and the babies who don't. It becomes a pretty straightforward diagnostic algorithm. At this point, for babies who have a mutation that they are likely a male who has x-linked ALD or a female who is a carrier. If there is not a mutation found on the screen, in theory, it could become a very complicated evaluation where they need multiple tests done in order to get to a diagnostic point.

Next slide please. Let's look more closely at a baby boy with a mutation in ABCD1. This is looking more closely at what you would have seen on that diagnostic algorithm. This would be a baby boy who needs to have another sample sent into the newborn screening program, as well as very long chain fatty acids ordered. Both of those are to confirm that in fact the correct baby was brought in to see the metabolic specialist, as well as to verify the finding in the screening laboratory. The maternal sample is also collected if the mom consents to have carrier testing.

When that gets to our lab, both Joe's lab test the C26:0 LPC, as well as the molecular testing is done to see if mom is in fact a carrier. Additional at-risk family members for this baby boy are offered testing. If it's a sibling and we have a sample in our lab, and it's been the parent's consent, we will pull that sample and test it for C26:0 as well as the mutation.

The likelihood of that happening hopefully will go down over time as we will have tested siblings already, but in the beginning, there were many older siblings who had not been tested because it was a new assay. Those baby boys are referred to genetics, neurology, and endocrinology. As I've said before, they typically start with genetics, but in one case start with neurology, and then follow up with the appropriate specialist for additional management recommendation.

Next slide please. A baby girl with an ABCD1 mutation, her parents are referred for genetic counseling. A confirmatory sample and parental samples, of course with consent, are sent to the newborn screening program and testing is also offered to at-risk family members for that female. As Joe mentioned, we have had boys in the family who have been identified with undiagnosed ALD through their carrier female relatives who screened positive. For those of you who listened to the Secretary Advisory Committee Meeting or who were there, there was one gentleman who spoke about his personal experience with his niece being identified through New York State Newborn Screening.

Next slide please. I included a little bit of information about carrier testing in females. That's because this is a question that we've had ourselves here in the newborn screening program, as well as a question that has been posed to me by other newborn screening programs that are considering now adding this condition.

Typically, carrier testing in a minor female or in a minor in general, in this case it's female because it's x-linked, is something that is discouraged. However, in the newborn screening testing, it poses a challenge. It's logistically very challenging to screen only males through the program. With our sample volume each day, it would be an extremely challenging task for the accessioning group to separate and accession those samples separately and order different testing.

There's also a chance to identify other peroxisomal disorders in the female population. Even though that's not what we're screening for, that is still information that can be given back to their healthcare provider. Then, also, there's chance to identify effective but undiagnosed males in the family. It's information that can be gathered through screening both males and females.

Just a little subpoint that carrier testing for other minor female relatives. I talked about if there's a brother who was not initially screened because he was born before screening started, we'll pull and test his sample. We do not follow that same approach for minor female. We will not pull and test their sample because at that point, we can choose not to test the sample and it's not something that's happening as part of the screening process. There has been some clinicians in the state who have ordered carrier on minor female siblings and that has been to evaluate their suitability as an HTT donor in the future.

Next slide please. The next part on the right side of the diagnostic algorithm we looked up which I referred to as the more complex side are the babies without an ABCD1 gene mutation. I think Joe mentioned this already but if there's a baby in the NICU, our follow up staff does not wait for the molecular results to make the initial referral notification. That's made as soon as we get those results. That's going to change to our protocol and the lesson learned from screening that those babies have turned out to be a peroxisomal disorder and in cases that's been useful information for the NICU to have is they're trying to figure out what's going on with a sick baby.

For those cases, there's a couple of different possibilities. One of them is an unidentified ABCD1 deletion or duplication which would not be detected by our sequencing molecular assay or in other peroxisomal disorder like the Zellweger Spectrum Disorder. When the baby comes in for their initial visit, very long chain fatty acid and plasmalogen are ordered, additional molecular testing may be needed if they are considering an ABCD1 deletion or duplication to be a possibility. I can say that even though in theory, the diagnostic algorithm for this group can become very complicated, in practice in the last two years, we've really seen that the diagnosis has been straightforward based on the clinical presentation and that most of these babies have ended up having a peroxisomal disorder.

Next slide please. The end of short term follow up for New York is when they have a diagnosis reached for the baby. We collect from data on those cases through a diagnosis form. We collect the results of the very long chain fatty acids, the results from plasmalogen. If any fibroblast studies were needed, we

collect that. If any other genetic testing was done, and then also a description of the clinical findings. We are happy to share that diagnosis form with any other states that are interested in having that if they're considering furthering their screening tests.

Next slide please. For these next slides, I wanted to briefly touch on the surveillance protocol. Dr. Raymond talked about this as well. If you can see, we published some information about both the diagnostic protocol, surveillance protocol, and treatment guidelines. A group of metabolic specialists from across the state, as well as Dr. Raymond and several our staff here at the Newborn Screening Program, work together on developing these protocols before we started screening so that we have a starting point to ensure that every baby who's been positive was getting a minimum evaluation across the board that was the same.

An important point to make is that ongoing evaluation of this protocol is very important. In fact, we've already some changes. This group that worked on this has met a couple of times both in person and on the phone to discuss the actual practice of implementing the protocol.

Next page. The surveillance protocols that the group came up with are at different time frames. First, at the time of diagnosis. Then, for asymptomatic boys and childhood. Then, for asymptomatic men and after age 18. It's important to note that once a boy or man develops symptoms of adrenoleukodystrophy that this protocol would not be something to follow anymore. That would be based on what their clinical status and their symptoms are at that time.

Next slide please. At the time of diagnosis, entering the practice for endocrinology and neurology, as well as receiving genetic counseling is important, as well as having some baseline endocrine labs done. The endocrine group in New York State is working closely with us now to look at interpreting those results in the newborn, as well as treating and looking at what the best testing would be. We may have more to come as that group continues their discussions now that they've had these 18 boys that they've started ordering testing on.

Next slide please. For asymptomatic boys, it looks more complicated than it is but during childhood, they need to have labs done for endocrinology and see their ACTH and cortisol every six months, as well as meet with their endocrinologist at least once a year. Then, they need to have a brain MRI done initially as early as 12 months. That's a change.

Initially, this protocol said six months, and we've learned through talking with the metabolic specialists that 12 months, in fact, will work much better for them, and not miss cases of boys who would not a transplant sooner. Then, have another MRI done at each 24 months. Then, from 36 months to 10 years to have more frequent MRIs because of the higher chance to develop cerebral disease

during that time. Then, to have an annual MRI done thereafter. Then, to continue meeting with genetics for counseling and evaluation as needed.

Next slide please. Then, even though there's quite some time before this is in practice, we wanted to be able to discuss with families what they could expect if their son in fact did not develop symptoms of ALD before he turned 18. That would be to continue meeting with endocrinologist every year and have annual labs done, as well as continue to meet with them or the neurologist can have an MRI done annually as well.

Next slide please. Dr. Raymond went over this well so I'll only touch on it very briefly that a transplant is recommended during early stages of disease due to the mortality rate. A lesson learned for us is that an MRI should be read by a neurologist specializing in ALD before going to transplant. The ALD MRI score should be greater than 1 and less than 9, and a performance IQ of greater than 80. If these criteria are met, that's a boy or a man who should be referred for a transplant or at least considered.

Next slide please. Just quickly, the molecular testing consideration, can the screen be performed without molecular testing at the newborn screening program? As Joe said, it's not intended to reduce the rate of referrals. There are benefits that we've seen through the process. It can reduce the diagnostic odyssey for the family by allowing for a targeted counseling session at the initial visit. It reduces the time burden for the clinician so that every referral that comes in doesn't need to have as many higher authorizations and just long drawing out timeframe to having an answer for the family.

Also, it allows for rapid identification and testing of at-risk family members. We've had boys who are between that high-risk 36 months to 10-year age frame where even though it wasn't an emergency for the boy who initially screened positive, for his brother, it's very important to move quickly. It also provides information on the performance of the screening test to the newborn screening program. We can look at, as Joe has showed you, this great data of how many of the boys who screened positive in fact had a mutation in the gene.

Next slide please. Just a few quick genetic counseling considerations for part of the process of management, it's important to consider that family members can be identified and should be counseled now that we are talking about an [inaudible] condition. Joe also mentioned that we did have a male with Klinefelter Syndrome.

There's also a chance to find females with Turner Syndrome who in fact have ALD, as well as a chance through carrier testing to identify non-paternity. Identification of the carriers and males with AMN leads to some psychosocial considerations like grief, anxiety, depression, despair, as well as consideration for things like life and long term care insurance. The group of genetic counselors across the state who are meeting with these families decided that as a group, we

would only give results to fathers with AMN in person. It's part of our plan to address this concern.

Next slide please. I'm running out of time but I just wanted to show you a hypothetical family where the arrow is would be the initial boy identified through screening and his mother is one of three siblings. It could turn out to be a family where her brother actually has ALD or more likely AMN and not been aware of that. Her sister is a carrier and has a son who was not previously aware of his diagnosis as well.

As you can see, a family, it can become quite complex and take quite a bit of time in the clinic to work through the family history and at-risk individuals was carefully considering things like life and long term care insurance, and as well as the chance that there's individuals who will in fact develop disease but are asymptomatic, as well as providing a lot of support to the family because they need to be talking with their family about the initial diagnosis and their newborn by the time when they're also having a newborn and providing a lot of support for that process is important.

Next slide please. Very briefly, long term follow up is something that we believe is really important. There's a lot of steps that need to happen for us to do that but we did work as a group to determine data elements and are close to being able to collect long term follow up data on the boys who are identified with ALD through the screen

Next slide please. We also developed educational materials. We have separate materials for the different scenarios on our algorithm. If you can see, this is a baby boy who has a gene mutation and the information is targeted to that scenario. We are also happy to share any of our educational materials with other newborn screening programs or clinicians.

Next slide please. I just would like to acknowledge the group that works on developing all of these protocols and guidelines, and as well as Dr. Raymond who work very closely with us. Next slide. Thank you so much for you time. There's my contact info. Right now, we're saving questions for the end but also feel free to get in touch with me if you have any questions after the call. Thank you all.

Patricia Hunt: Thank you Beth. We will now move on to Dr. Chris Haynes. Thank you.

Laura Russell: Hi everyone. Looks like we're experiencing some technical difficulties. We'd be happy to take questions at this time. You can press star seven to unmute yourselves and you can also type your questions in the chat box. We do have a question in the chat box. It's for Joe. Do you know where Perkin Elmer is with their kit?

Dr. Orsini: No, I do not. I have not gotten an update. I think it's something by year end but I wouldn't be quoted on that.

Patricia Hunt: Hi, Joe. This is Patty. I wanted to ask, are there any unintended consequences that you identified through your experience so far?

Dr. Orsini: I think one of the things that have come up for us and Beth eluded to it as well, I think the second tier test, the accuracy of the second tier test puts us in a little bit of a bind, I guess, because if we measure the average individual has concentration of marker at around 0.07 to 0.1, somewhere in that range, we ended up with this presumptive positive range being somewhere 0.24 to 0.4. Sometimes, we will see females with markers that's elevated but it's not within that cutoff range. I know that's a little bit of ethical dilemma that we have. That's one thing.

The other unintended consequence that's more affecting the docs on the outside is families are anxious when they have a screen-positive boy. It's almost the opposite of what you expect. They're trying to push to get early treatment and that can be problematic for them. I think it's one of the reasons why we're moving towards doing MRIs not until a year of age and not having it sooner is just to recognize the natural history of this disease and the individual not developing symptoms that would require early treatment. That's one of the more tricky things that we didn't expect. I don't know if Beth has any.

Dr. Haynes: Thank you APHL for giving me the opportunity to present my slides. Next slide please. I just want to give everyone a little bit of insight about how we prepare the QC and PT material for X-ALD here at the CDC. We start with a positive red cells. We buy this from Tennessee Blood Services. We wash the red cells with saline. Separately, we filter and sterilize our serum, and combine those two products, and we just hematocrit the 50%.

We then freeze that blood at minus 20 for a week or two. What this does is it burst all the red cells. We get a product that we call life blood. We bring that out the day before it's going to be spotted. The filter paper that we prepare this on is either Whatman or Ahlstrom. Then, we buy our Lyso PC Reagents from Avanti Polar Lipids. We buy them as powders and prepare them in methanol at about 1 mg per mill. I'll have a little more to say about that concentration in a moment. Then, we dilute it down to 0.4 micromolar for comparison to the internal standard and that's a stop check to make sure that we've prepared our stocks correctly.

Next slide. Thank you. Some of the lessons we've learned along the way are that you really cannot prepare these stocks at a higher concentration than 1 mg per ml if you're using methanol. You can make them at higher concentrations in chloroform but chloroform added to blood is not a pretty picture, and it didn't lead to blood spots that were acceptable. Even at 1 mg per ml with methanol at stocks, LPCs have to be added slowly drop-wise to the blood with vigorous starting to prevent clots, and you have to stir while you're spotting on the paper to ensure homogeneity from the first to the last card during production.

Next slide please. For quality control, we're currently producing three levels, no enrichment, 1 micromolar, and 5 micromolar enrichment. These enrichment levels are set by discussions with participants. As the needs of this program changes, we are very open to discussions about what those concentrations are. Quality control is 15 spots per card, 100 microliter spots. Proficiency testing is 5 specimens. We make 250 cards and each one of those cards have 10 spots, and the spots are 75 microliters.

In both cases, the material first gets homogeneity testing where we pull cards at regular intervals through the batch, punch multiple punches per card, and test them all at once. We then do excel scatter plots and fast analysis to satisfy ourselves that there is no significant change in these analyte concentrations from the beginning to the end of the batch.

Next slide please. Then, characterization begins once we've determined the homogeneity is adequate. Quality control is 20 independent runs. Proficiency testing is 10 independent runs. All of these are on set to pass based on previous logs of QC that are on the same plate. After we've accumulated the data, we prepare certification sheet which has averages, SDs, and confidence intervals in the case of QC and simply averages for proficiency testing. Right before we send the proficiency testing material out, we do a pre-distribution verification. This is done less than 30 days before shipment to make sure that the material has not changed in any way during the storage. We have to get the result that's similar to the original certified value.

Next slide please. Quality control and PT are currently distributed three times a year, and we simply pack the cards into Mylars, put the Mylars into tight vacs with paperwork, and ship those to view to the participants. In the case of quality control, participants receive the cards and the only price that we ask is that you share data with us. We ask for 5 analysis on 5 different days, each analysis we then duplicate, and we ask that that data is returned to us within three months of getting the package.

PT, on the other hand, we just ask for one analysis and we ask that that data is returned to us one month after shipment. We compile all this data into your annual report. The annual report is a really great resource for participants to compare their results to those of peers who are using the same method. I do have the link to our annual report here at the bottom.

On the next slide, I am going to share with you some of the data fourth quarter for 2015. This data is specific to quality control on this slide and it's all about the 26:0 Lyso-PC biomarker. In this case, it's the number of punches, not the number of labs. If you remember, we asked for two punches of QC on each of 5 days. When you see N50, that's actually 50 labs, and a N40 is 4 labs. First thing we do with the quality control is to divide it into bins based on the methodology that the lab used to get their data. You could see the first thing we did with the QC data is divide it into HPLC MS/MS and flow injection analysis MS/MS.

In the interest of time, I am just going to point out on this slide that if you look at the mean value for the unenriched lot, as Dr. Orsini has previous mentioned, the LC method gives a much lower value for the Lyso-PC 0.06 micromolar than does the flow injection method which gives you a value of 0.26 micromolar. We believe that has everything to do with this unknown compound that shares the same MRM tear in positive ion mode, and you simply cannot resolve that unknown compound when you're doing flow injection analysis. The two enriched lots, 14102 and 14103, you can see that both methods give you values that are similar to the enrichment of 1 and 5 micromolar, and again the flow injection analysis method gives you a slightly higher value.

Next slide please. Here are some results for PT data from quarter four of 2015. Again, we're looking at 26:0 Lyso-PC biomarker values. Let me bring your attention to the first column which is the expected value. This is the value that we got here at CDC after running this material 10 times with a negative ion mode HPLC method. I believe you can see that specimens 1, 4, and 5 are unenriched, specimens 2 and 3 are enriched. If you take a look at data returned by the 5 labs who participated in PT event, you can see that labs A and B used flow injection analysis and labs C, D, and E used HPLC.

The labs doing flow injection analysis got results that were similar to each other for unenriched and the enriched specimens and of the labs doing HPLC, I think you can see that lab C and lab D are potentially using different ionization modes. It looks like lab C might be using positive mode and lab D might be using negative mode as you can tell from the unenriched specimens. Lab E on the enriched specimen seems to be returning higher values than the other labs but again, this is the value of our annual report where you can compare your performance to peers running the same method, and then communicate with them, and figure out some of these underlying reasons.

Next slide please. Just to wrap up, these materials are available upon request to any domestic or international lab. I encourage you to look at our annual report data when you're trying to put your personal labs data into perspective. My job is to get you technical assistance with analytical methods and provide you with dried blood spots. I've got some stability studies published on these compounds. Coming soon to our dried blood spot material for X-ALD are the 20 and 22 chain lengths. I'd be happy to answer any questions at the end of the webinar. Thank you.

Patricia Hunt: Thank you so much, Chris. Because of a technical delay, we'll extend the QA session by about 15 minutes. If you hang on with us, we can provide you information at the end about P.A.C.E. credits. Thank you.

Laura Russell: We do have a question at the chat box. It is for Joe. Do you have data on patient mean, median, and standard deviation for second tier HPLC tandem mass spec method?

Dr. Orsini: The question was do we have …

Laura Russell: Do you have a data on patient mean, median, and standard deviation for second tier HPLC tandem mass spec method?

Dr. Orsini: I am not exactly sure of the question but if it's for all of the patients, all the ALD boys, I'm presuming yes, we do have the second tier data across a couple of … If it's just from our second tier lab, then we have it. If it's coming from diagnostic testing, we'd have it but they're coming from a couple of different labs. We can share that. If somebody wants to email me, we can provide that data.

Laura Russell: Thank you, Joe. We do have another question in the chat box. Any race or ethnic preponderant for the disorders?

Dr. Raymond: It's Dr. Raymond. No, there is no race or ethnic predominance. You just have to stop and think about it's an x-linked disorder and has apparently high mutation rates. Always, these ethnic groups are affected.

Laura Russell: Great, thank you. We have another question for Joe. Has anyone tried to measure very long chain fatty acids and blood spots as a second tier?

Dr. Orsini: The Connecticut Lab considers the negative ion HPLC method as second tier. They're running all of their samples through that method. I consider the answer to be yes.

Laura Russell: Great. Thank you, Joe. We have a general question for all the speakers. Is the CX-ALD method reliable for screening and identifying Zellweger's?

Dr. Raymond: I would say yes. If the elevation is a very long chain fatty acid, it's even higher than an ALD, it's reliable for the peroxesomal disorders that have elevations and that have defects in beta oxidation.

Dr. Orsini: I think probably we could say we have no reports of having missed any. If somebody were diagnosed outside of what we've detected through screening, we haven't been made aware of any such cases. That would support Dr. Raymond's reply.

Laura Russell: Great. Thank you. We have another question for Joe. Have you ever considered gender-specific cutoff?

Dr. Orsini: No, we have not. It's an interesting question. We've seen some females with very elevated marker which is surprising. Yeah, we have not done that. It might be a consideration.

Laura Russell: Thank you, Joe. Are there any other verbal questions at this time?

Patricia Hunt: This is Patty again. Question for Beth, since there is a high degree of picking up Zellweger's, do you prepare then materials for Zellweger's for New York Screening Program genetic information or educational material?

Beth Vogel: Yes. We have educational materials for a baby who screens positive but does not have a mutation that goes through the possibility that they could either have ALD if they're a male or have a peroxisomal disorder, assuming that it's the first visit when they go in to see the metabolic specialist or the neurologist that they would be given that material because at that point, in theory, we still don't know what their diagnosis is although it's worked out that in most cases, they are symptomatic and have a peroxisomal disorder. We didn't develop specific Zellweger Syndrome educational materials but stopped at that point of what they would need when they go in for the first visit.

Laura Russell: Thank you, Beth. We do have another question in the chat box at this time. A Participant is curious about why collected repeat blood specimen after sequencing when you are confirming this in plasma and very long chain fatty acids at the genetics clinic?

Dr. Raymond: I can take that. Primarily, because we're also getting the parent data. If it's a boy, we're going to get … I think Beth mentioned, we're going to get mom sample. If it's a girl, we're getting mom and dad and helping to trace that and also to confirm the identity for that child just to be sure on our end that we've got the right child.

Dr. Orsini: It helps us to match everything up and show clear picture for that family.

Laura Russell: Great. Thank you, Joe. Do we have any other verbal questions at this time?

Patricia Hunt: I'll ask one last question. This is Patty Hunt again for Chris. Normally, CDC sends out two QC shipments and you're sending us three for X-ALD. Is there a stability issue or what is another reason that you might be shipping three times?

Dr. Haynes: I'm sorry if I gave that impression, Patty, that we do three shipments a year. The QC, as you said, is only two times a year, one is in January and one is in July. I perhaps didn't make that very clear. Then, the PT is sent out three times a year, January, July, and October, unless you're a participant in our Quarter 2 UDOT event but X-ALD is not currently part of the quarter two PT distribution. Two QC distributions and three PT distributions.

Patricia Hunt: Okay, great. Thank you. I might have misunderstood, Chris.

Dr. Haynes: Actually, Patty, to get back to your question about stability, I have some publication about the C26:0 chain length that it's stable for at least 18 months in dried blood spots if stored with desiccant,, and I am currently doing a similar study on the 24 chain length.

Patricia Hunt: Great.

Laura Russell: Thank you, Chris. Are there any other questions? There are none. Patty, do you want to move on to P.A.C.E. credit information?

Patricia Hunt: Yes. Thank you, Laura. We are happy to announce that there is 1.5 P.A.C.E. hours can be earned from this webinar. Laura has placed the information on the slide and there will be a follow-up email for participants that can fill out. You'd need to fill out the survey within 30 days to get your 1.5 hours of credit.

This concludes the presentation but I wanted to take this opportunity to thank all of today's speakers for the excellent and insightful presentations on behalf of the QA/QC subcommittee, APHL, and our friends at CDC. This has been an excellent webinar and I want to thank the audience for participating today. As we all move forward with implementing these new disorders but information provided really is going to be very helpful. The presentation will be available at the APHL website and Laura will be sending a link in the next few days for the material.

I want to remind new folks to the newborn screening community that we do have several resources and previous presentations available for previous webinars that may be helpful for those joining the newborn screening community. Thank you very much and have a good afternoon everyone.