

SMA: Overview of Available Screening Methods

June 28, 2018

Transcript



Patricia Hunt: Hi, everyone. Again, this is Patty. Good afternoon and welcome to part two of a part two webinar series on spinal muscular atrophy.

My name is Patricia Hunt, and I'm a member of the Texas Newborn Screening Laboratory, and also a member of the APHL's Quality Assurance and Quality Control Subcommittee of the Newborn Screening and Genetics and Public Health Committee.

This webinar series is geared towards those who are or may be responsible for screening, diagnosing, and treating SMA. The series was developed with expert guidance from the APHL QAQC Subcommittee and in collaboration with the Newborn Screening and Molecular Biology branch at Centers for Disease Control and Prevention. Today's webinar will focus on overview of available screening methods for SMA.

The lines will be muted and recording has started already. All questions will be reserved until the question and answer session at the end. Everyone is going to be muted during the webinar.

Our first speaker is Francis Lee. He's a research microbiologist in the Newborn Screening and Molecular Biology branch at the Centers for Disease Control and Prevention. Go ahead Francis.

Francis Lee: Okay. Can I have next slide, please?

As many of you who participated in the first webinar know, SMA is the leading genetic cause of death in children. With a birth prevalence about 1 in 10,000, it translates to around 400 live birth with SMA per year in the US alone. Next Slide.

Many stakeholders believe that case or SMA newborn screening is compelling. Taking into consideration of the rapidness of progression in Taiwan SMA, the usual delay in diagnosis and the effective drug has been approved by FDA. Next slide.

The fact that Advisory Committee agree, so a recommendation was submitted to HHS secretary on March of 8th, and the deputy secretary promptly responds within 120 days as required by law. Next slide.

What newborn screening assay do we use? In the absence of a phenotypic biomarker, laboratory detection of SMA is mainly based on genotyping. Of the methods that have been used, we prefer real-time PCR for newborn screening, based on several different reasons. Next slide.

The first line is real-time PCR allows for high-throughput screening. Then, many of the newborn screening labs are already using this method for SCID, which means A, labs already have the lesser for the instrumentations and B, staff is familiar with the technique already.

I think the most attractive factor is that SMA assay can be multiplexed into the current TRI assay. This way that this new course to the lab in adding SMA to the TRI assay can be low as 15 cents, which is the cost of the SMA primers and probe. The new assay can replace the current standalone TRI assay. The lumbar pass you have to run remains the same, so there's no additional labor cost. Next slide.

There are currently four different versions of SMA real-time PCR assay being used in newborn screening labs. New York in hospital-base research project is using method published by Maranda et. al in Clin Chem in 2012. They're using an MGB probe, which I will go into a little bit more later.

CDC has developed two versions of the assay. Both versions are being used by state labs. PerkinElmer has also developed a different version, which is really similar to the CDC version, too. And the Indiana Newborn Screening Lab is adopting the PerkinElmer assay. Next slide.

They are some common challenges in designing a real-time PCR assay for SMA. Next slide.

Now, the assay has to detect the absences of SMN1 its own sequence. The major complication come from the presence of SMN2 gene in the human genome. SMN2 is a weakly functional gene but that is almost identical to SMN1. There's only one different nucleotide in its own exon 7 in position c.840, which is a C to T transition. Any method to detect the absence of SMN1 must avoid generating signal from SMN2. Otherwise, you might need to force negative results.

This essentially means we need to have assay that can detect single nucleotide polymorphism. Regular primer and pro-PCR primer will not do it. Next slide, please.

We find the solution in using LNA, the locked nucleic acid. LNA are modified ribonucleotides which can be used to substitute regular nucleotide to generally enhance level of binding specificity and LNA primers and probes can be ordered from multiple commercial sources. Next slide.

Initially, at CDC, we developed an SMA assay parted in the intron 7 of the SMN gene, it's because their sequence in the intron 7 are easier to work with and we also believe that the deletion or gene conversion of SMN1 always involve both exon and intron 7. Next slide.

The result from a Taiwan study recent issue, the folks in Taiwan found that over 15 sample tests are positive in the first-tier screening assay based in intron 7. Only seven of them will confirm in a second-tier assay based on digital PCR and MLPA.

The other eight cases were due to, next slide, please, a recombinant hybrid gene. In rare occasions, SMN1 to SMN2 recombines by crossing over. That would generate first a hybrid gene with SMN1 exon and SMN2 intron. This gene will give false positive result in assay based on intron as so many in the Taiwan cases. The other hybrid gene with SMN2 exon and SMN1 intron will give false negative results which poses a bigger concern in the screening assay. False positive can be rectified in the second-tier assay, false negative, we need to this case which we try hard to avoid. Next slide.

We wipe out assay, this time focusing on exon 7 as the main target. To gain maximum exclusion of SMN2, we use LNA in both the reverse primer, highlighted in yellow here, as well as the probe highlighted in green. This assay has two layers of specificity to eliminate any cross reaction to SMN2. Next slide, please.

This assay is super sensitive because it don't get any background from SMN2 but it will require a second-tier confirmation assay to exclude the hybrid gene from the SMN2 intron as so many of the Taiwan cases.

Massachusetts lab choose this assay thinking a second-tier confirmation on the small number of positive sample is not the issue and delayed the sensitivity. Next slide.

Now, using LNA primers is technically more demanding. You need to have good quality of DNA extract and some commercial mastermix won't work with LNA primer and your instrument has to be accurate as far as the temperature is concerned, but as long as your lab with this requirement it works fine, as in Massachusetts. Next slide.

But to give newborn screening and adoption that will soon require a second-tier confirmation, we've developed another version of the SMA assay. In this version, in reverse primer is now relocated to exon 7 so it does not involve the intron at all but to compensate for the loss of the second layer of specificity offer by LNA primer in version one, we further optimize the pro form maximum destination of SMN2. Next slide.

My colleague at CDC, Kristina Mercer, used every trick in the book. She used a short length probe, she put the location of the mismatched nucleotide in center of probe, and she used a triplet LNA substitution but I think the most important modification she did was moving the probe to the forward strand of DNA so that the LNA substitution is a pyrimidine, not a purine acid in the reverse strand. This does the trick. Next slide.

When we stress test this system using the equivalent of 1,000 copies of SMN2 per cell, the normal range being from being from zero to eight, so this is a rapid excess of SMN2. We still do not get any signal from SMN2. Next slide.

Now, certify with the validity of this SMN1 assay, we multiplexed this version into the current TRI assay for SCID. The results were exactly as what we expected. Now, in the first panel, normal newborn, we see signals from all three packets to try in green, the RNA speed in red, and SMN1 in blue.

In the bigger panel with SMA sample, we missed the blue SMN1 curve. With the SCID sample on the right, now we missed the green track curve. Next slide.

Then, we pass a panel of clinically-confirmed cases provided by Biogen. This table shows only the SMN1 part of the results. As you can see, the multiplexed assay currently identify all the patient samples. In addition to this, we task the 400 normal cold blood DBS samples. The results were all negative. We also compared with cells from a panel of 76 clinical samples provided by a clinical diagnostic lab for SMA and our results match perfectly with theirs. Next slide.

Since then, both version of the CDC SMA assay have been transferred and validated in three state newborn screening labs. Massachusetts is using the version one. Utah and Minnesota are using version two. I believe that a representative from this lab will present the data later in this webinar. Next slide.

Here are some thoughts I have about the SMA screening assay. The SMN1 assay is really the first newborn screening, first-tier assay that's based on genotype alone. The assay is highly specific in binding in order to avoid false negative result due to cross-reacting to SMN2.

However, with this level of specificity, any unknown single nucleotide variant is likely to fail to react. It's possibly give false positive result. We haven't encountered one yet. We believe that they will be rare. Otherwise, you will be finding the literature but if and when we find them and identify non-pathogenic mutation in the future, we can add additional probe to cover them, perhaps in the second tier confirmation assay. Next slide.

Here are items that we can provide from CDC to support the newborn screening labs. If the state newborn screening lab decide to try the CDC assays, we will provide reagent enough for assay development. We will provide testing

materials and technical support. We will also give you the primers and probe sequences so that you can order your own in the future. We can also provide hands-on technical training at CDC if you ask. Right now, we are making SMA positive QC driver spot materials prepared from patient cell line, spiked into leukocyte-depleted blood.

Lastly, we have just started SMA pilot proficiency testing in June 6th, 2018. 10 labs are already participating. We just get the first few of the results submitted. They all look very good. Next slide.

This map shows the current SMA screening implementation status in this space. The green one, three of them, are already screening. The dark blue one stays have completed assay validation and I believe they are ready to go. The pre-blue states are at planning stage. They have contacted us. We will supply support that they require. Next slide.

This is to acknowledge the many, many co-investigator in this project. Thank you for your attention.

Patricia Hunt:

Thank you, Dr. Lee. We will now hear from New York, Massachusetts, Utah, and Minnesota on their state experiences with screening for SMA. The seekers will be Dr. Michele Caggana. She's the deputy director of the Division of Genetics and the chief of the Laboratory of Human Genetics and director of the newborn screening program at the New York State Department of Health at Wadsworth Center.

Following here will be Anne Comeau, who's the Deputy Director of the New England Newborn Screening Program and a professor in the Department of Pediatrics at the University of Massachusetts Medical School.

Then, following Dr. Andy Rohrwasser is the director of the Newborn Screening Laboratory and serves as the Deputy Director of the Utah Public Health Laboratory. Andy's trained in human genetics and holds an MBA.

Then, followed by Carrie Wolf. She is a Newborn Screening laboratory supervisor of the molecular and core unit within the New born Screening Program at the Minnesota Department of Health and Newborn Screening Laboratory. Thank you.

Funke:

Hi, Michelle. At this time, can you share your screen by clicking on the share button at the top of the screen? Press star seven to unmute yourself as well.

Michele Caggana:

Hello?

Funke:

Yes. We can hear you.

Michele Caggana: Okay. We're having trouble here with our firewall. I cannot accept being a co-presenter, so can you put my slides up and I'll just tell you to click through? I'm on Denise K.'s computer right now because mine actually won't even view the slides. I can't even see the webinar from my computer. I love technology.

Funke: So do we. ☺ I think at this time, actually, we're going to go ahead and go with Anne and we're going to go to your slides right after this.

Michele Caggana: Okay, okay.

Funke: Anne, if you don't mind pressing star seven, we're ready for you.

Anne Comeau: Okay. I think I'm ready for you. Thank you for the opportunity to talk about our experience in Massachusetts, which is a little different. If you could go to the next slide, please.

I just need to provide this disclosure. The medical school holds the intellectual property. Next slide, please.

Francis already covered reasons for screening for SMA so I'm not going to reiterate. Next slide, please.

I did want to thank both Doctors Lee and Mercer for sharing their protocols with us. The people in my laboratory who optimized the assay for our purposes are Lan Ji and Jenn Navas. Next slide, please. Next slide, please. Oops. Yeah. Okay. Next. Yes. That one is great.

As Dr. Lee indicated, the assay that we are using is a two-tier assay. Not only that, but the assay that we're using is focusing only on SMN. We are not doing the TREC assay for the following reasons. Many of you know that the Massachusetts program runs optional screening, which is consent-based, and our SMA is consent-based. Furthermore, and probably more importantly, the New England program provides screening assays not only for Massachusetts but for Maine, New Hampshire, Vermont, and Rhode Island. We certainly want to provide only one trick assay for everybody.

Until all states and until all of Massachusetts is doing SMA, we cannot possibly screen for both. We cannot possibly multiplex the assay. In order for us to multiplex the assay, that would mean that we would be running one SCID assay for some of our population and another SCID assay for the rest of the population. We are currently running an SMA assay that is independent of the SCID assay. It's working fine.

The assay that we're using is, as I indicated, two-tiered. It is designed to not detect carriers, only to detect the affected. As Dr. Lee indicated, we're using the assay that has an LNA-modified reverse primer and an LNA-modified exon 7 probe, which is also very sensitive because of the length of the amplicon.

In optimizing the assay for use in our laboratory, tier one was designed so that the amplification of the SMN1 exon would be pushed to its limits in comparison to our internal control of RNase people. The typical CQ that we have for the exon 7 in this tier one is about 31, whereas the typical CQ for the RNase P in the same assay is 25. That allowed us to have a clean call. We are only calling undetermined for this.

Specimens that show undetermined or SMN1 with a good RNase P go on to tier two. The tier two, the CQs that we're seeing for exon 7 and for RNase P are similar. They're more like a CQ of 25. With this second tier, we will sometimes pick up that SMN1 hybrid. Next slide, please.

During our validation of this assay, we ran about 3,000 de-identified specimens and even though typically we wouldn't have all of them running on tier two, we ran about 3,000 specimens with the tier one and tier two assay just to know what the CQs would be like. We also ran the set of pre-characterized specimens that we purchased from Coriell. We had specimen from the CDC. We also had a variety of specimens from Biogen, which were particularly helpful because they included both the SMA patients and obligate carriers, their parents. With the validations, we were quite happy. We've run these samples several times but on validation, we had 100% pass. Every time we run these samples, we're at 100% of what our expectations are. Next slide, please.

Just to indicate as we typically do in Massachusetts, we have a clinical working group set up. The clinical working group people that we have include two neurologists, Dr. Darras, who runs the muscular dystrophy clinic at Children's Hospital and Dr. Swoboda, who is a neurologist at MGH. In addition, we've brought on geneticists from around the state. The plan is that when we have a positive screen that these people will be on the front lines dealing with the positive screen in order to have a very quick turnaround. Next slide, please.

We began screening with babies who were born January 27th of this year, so January 29th, we were live in the lab testing specimens. Since that time, we tested a little over 20,000 babies for SMA. I had indicated before that SMA, like all of our pilots is done with consent. We have, now we're at about 80% of parents who consent to SMA screening. Next slide, please.

We have a very small number of babies that prompt tier-two testing. It's very easy for us to handle. The handling fee SMA and the SCID side by side has not been a problem. The assays are good. They go smoothly. The flow in the laboratory is fine. As I indicated, we have a small number of specimens that prompt tier two. The small number of specimen that prompt tier two when we have one, the tier-one assay comes off in the morning and we can usually get the tier-two assay off by the end of the morning. Having tier one and tier two is a pretty good laboratory flow. Next slide, please.

Of the babies who went to tier two, most of them turned out to not be SMA. We did find once that turned out to be an SMN1 hybrid and the rest showed to

be normal on tier two. When we do this, we are usually rerunning tier one and with tier two. I'll speak a little more about that in a following slide. Next slide, please.

Most of the babies who prompt tier two in Massachusetts have been NICU babies. In the next slide, please, you'll see, we did have one positive newborn screen that turned out to be a false positive. It's false positive in that this specimen turned out to have an inhibitor. This baby had a tier-one assay that was undetermined, went for the exon, on tier two was undetermined for the exon and the intron. As part of our protocol, we ask the invasive specialist to send to us a new filter paper. They did and that turnaround time was very fast. We got the result of that new filter paper from the baby who was seen by the subspecialist very quickly. Totally expecting to see another undetermined. It was perfectly normal.

Basically, I was able to take this specimen and combine it with some normal specimens. We're quite convinced that this was one of those NICU specimens that had, it was drawn in the NICU that was actually had some heparin or some other inhibitor in it. It was enough to shut down the other specimens. Next slide.

I think that's it, actually.

Funke: Yup. That's it.

Anne Comeau: Yup. That's it. That's our experience. Thank you very much.

Funke: Thank you. At this time, we're going to have Michele Caggana present. Michele, bear with me one moment as I share your slide.

Michele Caggana: Okay. Can you hear me?

Funke: Yes. We can hear you.

Michele Caggana: Great. All right. Thanks for bearing with me through this. It was a flurry of activity here trying to figure out how we were going to pull this off without having it show on my computer.

Anyway, I'd like to thank APHL for asking me to visit the QAQC committee. I'm going to talk to you about two things. One is our pilot experience and then our preparation for form implementation for SMA screening. Next slide.

I'll just tell you to click, I think. I don't know if they're all going to show or not. Okay. Biogen did fund our pilot study but they have not had any role in the analysis for data or interpretation or anything regarding patients and how they're handled after we complete the screening. Next slide.

As you know, SMA has many different types. This just shows you the range of phenotypes but primarily they have muscle degeneration due to lack of the motor neurons. This has already been discussed but in the interest of time, I'll ... Next slide, please.

Okay. You are showing automation slowly. The take home message is that the age of onset and symptoms and severity are very variable. Next slide. Next.

It's most common genetic cause of infant and toddler death. The incidence is variable depending on what you read and the expected carrier frequency is somewhere between one in 50 and one in 60, and, as you've heard, most of it is caused by the homozygous solution of the SMN1 gene, particularly exon 7. Next, and again.

And there's your deletion there, and again. Then, this just show you that there is one particular difference between SMN1 and SMN2. SMN2 is a non-functional protein that has a little bit of activity still. It's truncated. The number of SMN2 copy numbers can dictate what the phenotype actually looks like. This is what modulates the severity of the phenotype. Next.

This just shows you some information on the different types and again, SMN1 is mostly caused by the deletion copy. Then, that's okay, most patients have two copies of SMN2. That dictates Type I. Then, as you move through the various types, the number of SMN2 copies increases.

The next one shows survival curve. That shows you that SMN1 type with one copy is that steep curve, the survival curve. Then, you can see SMN3 is the large, thicker line and the survival is much better. Next.

The newborn screening for SMA has been talked about quite a bit. There was really no argument back in 2008 when it was discussed at the advisory meeting. There was a biomarker and there was a test available but it hadn't been tested in a high throughput setting. At that time, treatment was only palliative but since then, next click, you can see that there is an accessible treatment which we've all heard about and it actually has been shown to have quite a difference in the lives of these kids when it's administered, particularly when the kids are asymptomatic. Next.

As many of you probably heard, it was discussed quite a bit, the evidence review was done by the advisory committee. Our pilot data was used as part of the evidence review for that. There was a lot of discussion at the time that the vote was made whether or not to recommend SMA to universal screening panel. On February 8th, it was approved at an eight to five vote. The secretary hasn't chimed in yet and that was due June 9th so almost two weeks behind in whether or not the recommendation is going to be made so that it is officially added to the panel. Next.

The pilot screening program that was conducted in New York was with Columbia Presbyterian and Columbia University Medical Center. There were three hospitals involved in the study and they're shown on the bottom there. They had a moderate number of births in each hospital. The goals of the study were actually developed and validate the SMN1 assay in our laboratory demonstrate that we can do it in a high throughput setting so that it was feasible for newborn screening to satisfy the advisory committee's concern.

We offered it in a consented process. We wanted to access the uptake, what the outcomes were and we wanted to access carrier status both in frequency and how parents and families felt about learning of carrier status. Next.

We had an opt-in model as required by our IRD. The three hospitals had about 12,000 births per year so fair number of births. We developed this short video that families and mothers could look at while they were in the hospital. We had coordinators at each of the hospitals that would answer questions, could describe the study. Consent was given either on a tablet or it could be a paper consent form, if they preferred that. That is information went into REDCap. We were able to check, get access to the REDCap system so that we that could compare what we received in the newborn screening program.

On the card there, you can see marked with SMA, we would separate those out. Then, we'd also compare the consents with the cards that were marked in the REDCap system. That told us that that baby would be screened for SMA. We also developed a brochure that was in the style of our newborn screening brochures, so that the parents could have more information and then, could take that home with them as well. Next.

Our assay is the one that was described by Anhuf in Human Mutation quite a while ago. We used, it's a deletion assay, of course. The DNA first test, we extracting it from the drug blood spot using the standard CHASM method that's in use in our lab for most other things that we do. We set up a TaqMan real time qPCR assay using RNase P as an internal control. We set up originally on the 7900s but we moved to the QuantStudios and we calculate the relative quantity using a delta-delta CT calculation. Because we had, as part of the study, carriers, we had it set up such that it would calculate the relative client assay we'd get copy numbers for 01 or two or greater. The algorithm is shown there on the right.

We went through this. We would report them just like a regular newborn screen. We would report carrier status. Parents also got a letter. If there was zero copies, they got a phone call. Then, they had access to genetic counseling if they were carriers. It was screen negative, the reports just went out like a regular, as an extra page basically on the newborn screening report. Next.

Our validation is shown here. We had 45 positive controls and most of them were from Biogen. I have them on a separate slide to show you. You can see on

the left side is stamped the amplification of the RNase P and on the right side is the SMN1 amplification. They're marked accordingly so the normal is shown by that blue box arrow where it's greater than one copy of SMN1. Then, zero copies of SMN1 is shown by the pinkish or salmon-colored box on that slide. I think you can click one more time. Yup. There, in early validation, we used 4,000 dried blood spots. Each of those is an RQ value and we did it in three replicas.

The probe and primer system that we used and the probes are shown below so we use a five prime FAMN for SMN1, five prime VIC for RNase P, and we used the minor groove binding non-fluorescent (I was going to say functional.) A, non-fluorescent quencher. And the mix contains a ROX standard. Next slide.

This just shows you the specimens from Biogen that were on multiple times and separated out so you can see what those reactions together. Then, you split them out, the RNase P and the SMN1 behave quite similarly in the amplification. Next.

Denis and Coleen put together a spreadsheet that allows the data to be merged and analyzed. It's done all on an Excel file. This is an example of what our controls look like. We have two calibrators that are done in triplicate. These are samples where we know that they have two SMN1 copies. Then, there's a zero copy standard, a one copy and a two copy control. Those are done and you can see the zero copy. If you don't get any quantity. Essentially, you get just the blank. Then, when you have a one copy, it's around .5, and when you have two copies, it's around one. It's color coded nicely so that when you analyze the data, looking at it after it's been brought by the text, you can see that exactly what to look for. Again, we calculate the relative quantity used in the delta-delta CT. Next.

This is what the assay results actually look like. I show you one equivocal here. We have two equivocal ranges in our assay. Then, there's also a copy of a heterozygote that was detected by our assay and the rest on this page are normal, so they would all be reported as screen negative.

For the study, we ran all of our specimens in triplicate, just to make sure that we didn't have any issues with the assay and it gave us the ability to look at how the assay behaves and it was just because it was brand new and we hadn't done it before so we've continued this entire pilot to run everyone in triplicate. Next.

We have a percent CV failure on those two equivocal ranges and then those would have to be repeated. Then, if you have the confidence limit, if they have too much variation between the triplicate, we would flag those. That's shown here in pink. In that case, we would repeat that sample as well. We also have its gained that we do DNA sequence only for exon 7. We don't sequence the entire gene. Although the different bullets are shown here for the reasons why we might do that sequence to look at either a little drop out or a look for

conversion type events. If we have a high CV2 times or we have equivocal or we get a positive result for a carrier or a zero copy for SMN1, we would run that sequence test. Next.

The result of the pilot study are shown here. Denise gave me the recent numbers. We've screened 14,089 infants. We've detected in that 200 carriers. Our opt-in rate is quite high. It ranged from about 91 to 93%. If you look at the different hospitals, you can appreciate that the carrier frequency is somewhat variable. It's also lower than the expected carrier frequency. That's because a lot of our carriers, we think, a lot of our individuals actually have that two plus zero genotype where they actually are carriers but we can't figure that out, use an assay we use because those show up as two copies. They have two copies on one chromosome and no copies on the other but our assay can't distinguish that. Our carrier frequency was actually higher or lower, I guess, than expected.

Our retest rate is about 1% but mostly the equivocal fall around the carrier calls so that's going to go away in live testing. In live, we're not going to have a CD calculation because we're going to be doing it in singlicate. Next.

Just a little bit about carriers. When we last checked, when we looked, we had 113 carriers and about 14.1% of those agreed to genetics but of those, a few made an appointment and even fewer actually came for the appointment. While most parents were concerned about carrier status after they talk to a counselor, they generally felt like they understood it better. An interesting thing was almost half, 43% or so, actually knew they were carriers due to prenatal screening. Those individuals were actually less concerned then because they probably heard the same thing a couple times. They understood the information better. While there was a lot of concern about carriers, it seems, at least at these hospitals, SMA uptake in the prenatal setting was quite high. Next.

We had one affected infant so far. When she was genotyped, she was homozygous for the exon 7 deletion. She also had two copies of SMN2. The natural history is shown there all of the things that would have occurred had she not been screen early. She was put on Nusinersen at the time of her diagnosis. Back then, it was still in the clinical trial and it has since moved to the FDA approval status. She's been tolerating the medication well. She's meeting all of her milestones on time. She runs. She walks. She crawls and talks. There's a couple of videos of her. It's pretty amazing. All right. Next slide.

This is her, actually her scan. You can see here where we detected that homozygous deletion on the amplification plot. We tested this child many times to make sure that we weren't going to call out a homozygous deletion. As some of you may have heard, we found this baby on, I think, the third day of testing, so we were concerned that we got no amplification that this could be a real one. She was lucky in a lot of ways. Next slide.

In conclusion, the pilot study, our screening, we showed that screening is feasible, that families want testing. Our denominator's higher. Our carry rate is lower than we anticipated. We've only found one infant and now one in about 14,000 babies. As I said, she was treated and is asymptomatic. Next.

As we prepare for population-based screening, we have a regulatory amendment in process. We also have a bill in New York that's pending so we're watching both of those move through the system. We're in the process of certifying our specialty care centers. There's 11 of them in the state. We decided that we would not report carriers and that we're going to multiplex with SCID and the qPCR assay and do that in a singlicate. We are also going to do the reflex digital droplet PCR only on the SMN1 homozygous solution. Next slide.

The assay as it stands now, for universal screening in New York, we're planning on using a CT cutoff rather than doing that calculation I talked about earlier. That way, we get just a positive or negative result and we don't have the copy number information so that we don't know their carriers are just going to be screen negative for SMA.

We have to change the probe system and we're in the process of validating that. The probes are shown there at the bottom right. We're using the quantum mix, which has purple haze as the standard. Okay. Next one.

As I said, we're not reporting our carriers. We're trying to figure out, I guess, naturally, also what to do with the leader onset forms and when treatment will occur. We are going to try and provide SMN2 copy number and the information is better with the CD PCR than it is with the qPCR so we'll be able to give that information.

Also, we're wondering how the incidence of SMA, is it going to be more common now that we're able to detect all forms of the homozygous solution, so that will come out in time as more and more states screen for SMA. As we know, we won't be detecting non-deletion mutations and we have to make sure that the report language, it's importantly to state that a child could still have SMA even if they're screen negative.

The advisory committee was very careful in saying that we were only screening for the exon 7 homozygous solution to help avoid that discussion of whether it was a "Missed newborn screen or not," so just making sure that we qualify what we exactly are screening for on our reports and in our literature.

Then, issues around treatment. We're talking about what are the long-term effects? Are babies still tolerating the medications? She hasn't shown any problems with that. I'm working with our Medicaid and both fee for service and managed care programs as well as private insurers to make sure everyone understands that this is needed quickly, that we can't wait for two months pre-

authorization type of request and so that can process as well. There's a lot of moving parts to this but we're hoping to screen by year's end. Next slide.

Thankfully, I have this in here because Denise is sitting next to me, let me use her computer but thanks to Denise for the slides and a lot of the data. Lastly, all of the people that were involved in the pilot study and in the laboratory. Thank you very much. Thanks for doing those slides. I appreciate it.

Patricia Hunt: Thank you, Michele. Andy, if you press star seven to unmute.

Andy Rohrwasser: Can you hear? Hello?

Patricia Hunt: Yes. We can hear.

Andy Rohrwasser: Okay. Great. Hello, everyone. This is Andy Rohrwasser. I will actually not give the presentation. Katelyn Logerquist and David Jones will present the laboratory work and the analysis work. I just want to give a huge thank you to Francis Lee from the CDC and all their work and resources they provided towards implementation of this assay. Yeah, and now, I'll hand it over to Katelyn but I will be available at the end for questions.

Katelyn L.: Okay. We use a PCR-based triplex assay, as described by Dr. Lee, where we use SMN1 TREC and for internal control, we use RNase P. Our extraction is fully automated. We use the TECAN Freedom EVO. We've had a pretty good experience with it. We have two regions that we use, a home brew of wash one with PPS in between 20. We also use hydrogen solution for wash and elution. We also with our automation go from a 96 well format to a 384 well format and for our real-time PCR implementation, we use the Roche LightCycler 480. Next slide, please.

Just to walk you through our extraction protocol, we first punch 3.2 millimeter punch. Then, we put it on the TECAN where the first wash we do, 80 microliters of the home brew solution where we just do an eight minute incubation while shaking the 700 rpm maintains to the whole protocol. We use the Inheco. We remove all the liquid or as much liquid as we can and then add the Qiagen solution to for another wash thing so that 80 microliters and eight minutes room temp.

Then, we remove as much liquid as we can, the last dispense, add Qiagen solution to. We put the temperature to 70 degrees Celsius and that is for a half an hour. The final transfer, we enter the 384 plate, we do 3.5 microliters of the crude DNA. That gives us a total PCR volume of 12 microliters. Next slide, please.

This is just a picture of the bed of our TECAN and what it looks like. We have it divided up into the extraction and the DNA preparations. Next slide, please.

This is the DNA extraction preparation, the 96 well plates where you can see the Inhecos. Next slide, please.

This is where the mastermix edition side is and where also, we do the 96 well plate to 384 well plate consolidation. You can see where the mastermix block is. We have the mastermix in the right-hand side. We have the tube with full positive control. Next slide, please.

We have full known positive controls that were previously run for our normal control. For our abnormal controls, we have known SMN1 and TREC, where there are dry blood spots that we punch and extract with the samples. Next slide, please.

This is the SMN1 filter with our amplification plot. You can see the red line of all of our patient samples. They're normal patients samples and are positive control is in there. The green line is the SMN1 negative control where you're seeing no amplification. Next slide, please.

This is the TREC amplification plot where you can see these are the same thing, normal samples in the positive control is in there and the green line also shows the negative control with no amplification.

For our validation for the SMA/TREC assay, we did a reproducibility study with pulled sample multiple times on multiple plates for within run, between run. There is little to no variation between runs for all analytes. We have a limited case control study which was blinded where we had, for SMN, we had known Utah, babies that were SMA positive and Biogen samples as well as with TREC, we had known samples for our skin. The population analysis, we ran 5,080 for SMA and 3,000 for SCID, which we'll show in a later slide. Next slide, please.

Okay. This is the SMA abnormal. As you can see, the origin, we have Biogen samples with some CDC controls and Utah samples that those were the babies that we knew were SMA positive. As you can see, for the SMN1 CTs, we had no amplification but we had amplification for the RNase P. These matched 100% with what we needed. At the bottom you see a normal sample just for frame of reference where the 25.8 CP for estimate. Next slide, please.

These are our SCID abnormal. As you can see, the first two were caustic SCID where we had no amplification. We also were able to pick out a variety of other diseases like the secondary T-cell lymphopenia and DiGeorge. There were two that we wouldn't have found for second T-cell lymphopenia, that being okay since our main focus are the first two, the caustic SCID and the AD SCID. On the bottom you can see we have a normal patient just for a frame of reference.

Andy Rohrwasser: Right. These next few slide are going over our population analysis so we perform for both SMN1 and TREC. We utilized a kind of statistical analysis tool to do a lot of our mean and centered deviation calculations as well as plot these

graphs so just want to plug that a little bit but here for the SMN1 so this is the first three months of data from our screening, basically from January until through March. They're about 5,000 infants that were screened during this period of time. As you can see with this particular figure, it's a pretty normal distribution with a CP mean of about 27.5. You can go ahead and go to the next slide, please.

Here we have the TREC population analysis. Within this plot we've analyzed 3,000 patients in this particular population analysis. Again, we see there's a fairly normal distribution with a little bit of tailing to the right. The TREC CP, the mean would be about 36 for this particular figure. Go ahead and go to the next slide.

Kind of do a count for that tailing to the right. We decided to, instead of reliance on CT value, to calculate a Z score and so essentially what the Z score is, is it takes an individual patient value for TREC. It subtracts it from the mean of the population for TREC, the CP value, and then divides it by the standard deviation of that TREC.

Really what you want to do here is you're looking at how many standard deviations below or above the population, mean, and individual patient is. You have to ensure to use a large population size to really establish those population means and standard deviations for your TREC EP. Go ahead and go to the next slide.

As you can see here, we plotted the Z score of the 3,000 patients and this data was sorted by the CP value for TREC and, as you can see, it really represents that normal structure that we notice within when we plotted just the CP values, we see that we have a really nice Six Sigma type distribution here for Z score. Go ahead and go to the next slide, please.

To establish our cutoffs, what we did was we, again, utilized our and we looked analyte by analyte. We looked at the mean and standard deviation. For SMN1, we have 29.15 with a standard deviation of 1.35. We then went onto look at the two standard deviations, a 99 percentile, the three standard deviation and the 99.5th percentile. For SMN1, we elected to go with the three standard deviation CP value of 33.2 as our cutoff. For TREC, as you can see, the mean was 36.98 with a standard deviation of 1.66.

Again, we looked at two standard deviation 99 percentile, three standard deviations and 99.5th percentile but, as I mentioned previously, instead of utilizing the two standard deviation or the percentile or three standard deviation, we elected instead to go with the Z score of 2.8. That would correspond with a CP value of about 41.65, which, as you can see from this table, would fall in between the 99th percentile and the three standard deviation just for reference.

We also looked at the RNase P as our housekeeping gene, which had a mean of 29.71 and a standard deviation of 1.39. We elected to go with a cutoff of the two standard deviation value of 32.49 for our RNase P. Go ahead and go to the next slide.

Here we have the breakdown of our actual workflow that we're following here. We have an initial screening for SMA where we assess whether it's normal or if it's a retest. For it to fall in the retest area, essentially you would either have no amplification of SMN1 or you would have late amplification of SMN1 or you would have late or no amplification of both SMN1 and the housekeeping gene. Then, if that occurs and you end up in that retest bucket, you would then proceed onto the confirmation on that first screen.

At this point, it would ever be resolved with a normal screen and indeterminate or go out as an abnormal. For it to be an abnormal, there has to be no amplification of SMN1 but amplification of housekeeping gene. For it to fall in the indeterminate zone, you would have to have no amplification of both SMN1 and the housekeeping gene or late amplification of both.

If that's the case, we then proceed onto a five-day collection of a second specimen, which we run a second screen for SMA and, again, it basically follows the same workflow except for that on the confirmation, if it has any issue where we see late or no amplification of SMN1 and RNase P, we throw it out to confirmatory testing at that point. Next slide, please.

For SCID, we've broken our workflow up into two different workflows, if you will. We have one for our term infants and one for our preemie population because they really follow different paths for really determining whether or not they have the disorder.

As you can see for SCID, we have a very similar workflow to what you saw previously for SMA. Essentially, we have the normal retest on the initial screen, which, again, follow very similar workflow and it just proceeds on down. Go ahead and go to the next slide because I want to focus on the preemies.

This one is way more complicated because really determining if a preemie has SCID is way more cumbersome and they're very unique population with SCID. Essentially what we do is if they're abnormal or indeterminate on the first screen on the confirmation, we put them into a C note bucket, which means essentially that we're just going to proceed onto a second specimen with them.

Once we get the second specimen and proceed onto the confirmation of it, they can be indeterminate or abnormal. We don't want to send the abnormal out for confirmation yet because oftentimes, our clinical specialists would like us to proceed onto the third screen and reassess. Both the indeterminate abnormal at the point of the SCIDRC, as you can see in the middle of this figure, they would proceed onto a third screen at that point. On the third screen is where

we actually resolve whether they're normal or abnormal and then would proceed onto confirmatory testing. Go ahead and go to the next slide.

We wanted to really take a look at what we've done to date with our SMA production data and, as you can see, we've screened about 16,537 patients. This data's from January 29th to May 31st. If we were to add in the June data, we'd probably be somewhere on the order of about 20 to 25,000 at this point, give or take.

Really, what I want to point out here is though we have a repeat requirement of about 5%. You can't really see that within the repeat first screen because the repeat first screen only represents what are lab information management system flags for repeat testing. Also, within that 5%, you'd have to consider any failed plates as well as any points at which the lab staff decided to intervene because they didn't really like the plot on the PCR instrument so they can also mask the sample. Again, we have about a 5% repeat requirement for SMA and SCID. It's also very representative of that 5% repeat requirement.

In terms of second screens that we've moved onto, we only had a total of 21 so a lot of those kind of repeat specimens are really resolved within their initial screen. We just had an issue where, on the initial screen, they ended up having no amplification of any of the analytes and so we proceed onto to do that confirmatory or confirmation screen where we see a lot of them resolve as normal and do not proceed onto a second specimen.

In terms of actual abnormal that we've seen, we saw a one plus one, which really wanted to highlight because we want to focus on that in the next couple slides, the one being a true positive and one being a false positive. Go ahead and go onto the next slide, please.

Our abnormal case one was a positive screen that was reported. It was assessed in clinic with no symptoms present. Confirmatory testing confirmed the diagnosis of SMA with zero copies of SMN1 and three of SMN2. This actual patient had a family history of SMA and predicted to be SMA type two phenotype. Go ahead and go onto the next slide.

This is the false positive case. We decided, after seeing the abnormal result from the screen, that we wanted to proceed onto diagnostic testing because we were really early in our screening process and really didn't want to wait for the repeat screen just because we wanted to be sure that this was either a true or false positive.

On the initial screen, we saw no amplification of any of the analytes and then on the repeat screen or the confirmation screen of that initial first screen, we saw a late amplification of SMN1 and then normal amplification of both the housekeeping gene and TREC. This kid was assessed in clinic with no symptoms present. Confirmatory testing showed two copies of SMN1 and one copy of

SMN2. This was confirmed in two independent laboratories. SMN1 was repeated, then, on the second newborn screen and we found it to be normal on the second screen, so a false positive. Go ahead and go onto the next slide, please.

Really, in summary, what we really wanted to highlight is that true cases from what we've seen show no amplification of the SMN1 as an analyte. In production assays, we can obviously, this works for both SMN1 and TREC. We've shown that we can multiplex the assay for both analytes. We've shown that there's a concordant performance between our TREC analysis with our LBT and the EnLite assay, which we currently run in production so it'll be switching off around mid-July. The 384 well format really allows for economies of scale. We successfully passed our initial PT for SMA and TREC using the LBT assay.

That's it. We appreciate your time.

Patricia Hunt: Thank you, Utah team.

Carrie, can you press star seven to unmute? Yeah.

Carrie Wolf: All right. Looks like my slides are coming. I'll introduce myself. My name is Carrie Wolf. I'm the lab supervisor, intermolecular and core units as a program. I want to thank Francis and Christina from the CDC for all their help during our validation process and help getting us ready for screening. We couldn't have done it without them.

I'm still not seeing my slides.

Funke: Right. Yeah.

Patricia Hunt: Carrie, just give us a minute. They're not showing up but we're re-uploading them so just bear with us one second.

Carrie Wolf: Sure. No problem.

Patricia Hunt: Okay. Can you see them now?

Carrie Wolf: Yup.

Patricia Hunt: Okay. Great.

Carrie Wolf: Great. You can go to the next slide. Perfect.

We decided that we wanted to multiplex SMA with our current laboratory developed assay for SCID. We decided that it would save money, analyst time, and that it would work out better. It created much larger validation in that we not only had to validate SMA, we had to revalidate SCID. We only detect

homozygous deletions of the SMN1 gene. We do not detect carriers. We also do not run SMN2 copy number.

We spoke with our specialists on whether or not they felt that would be important. They decided that they could easily ask for the testing and it would also confirm the SMN number, so then we could confirm that we have the right patient, that there wasn't any sort of patient mix-up and they could get the copy number for SMN2 within a good amount of time. The DNA extraction that we use is also used for our CF mutation analysis molecular assay or any other molecular assay that we might want to use. Next slide.

Similar to everyone else, we look for TRECs in the sample for SCID. We also use RNase P as our reference gene. We use something different for SCID cut-off where we use multiple of the median where we take our TREC CQ and divide it by our median. Our cut-off is based on that multiple of a median. We have found that to be very helpful. We validated using two different extraction methods. One that's super clean and one that's a little more dirty. We found that we can use the exact same multiples of the median cut-off for either extraction by doing that for SCID.

We multiplex our assay by looking for the absence of SMN1 so we add the primers and probes to that assay. You can see in the picture, that's what our normal samples look like. SMN1 and RNase P are very close to each other and TREC is separated. We have seen that, if there is an absence of SMN1, then we have a true positive SMA patient. Next slide.

Minnesota started screening for SMA on March 1st of this year. As of Tuesday, we have screened over 23,000 babies. We have not detected any undetermined specimen so we haven't had any samples where RNase P, SMN1, or TREC have not amplified. We maybe have had just single samples where maybe they didn't get enough DNA or there wasn't enough mastermix. Once we repeated it, it repeated fine. So far, we have had two confirmed cases identified. Next slide.

Our first confirmed case, the specimen was received on day four of life. Our positive results were reported on day six of life. It was clinically determined that the baby had three copies of SMN2. They also discovered that the newborn had a two-year-old sibling that was reportedly not walking so there was a lot of investigation as to why a two year old wasn't necessarily picked up or what's going on. "Have you seen a neurologist?" There was a lot of work to find out exactly what was happening. This shows a really good case of when we identify a baby with having a new disorder and we're first screening, we might be able to find a sibling that might have something.

Both the newborn and the sibling were tested clinically for SMA. They were found to have three copies of SMN2. The family now has two positive SMA children in their family. The treatment for the newborn occurred on day 67th of life. We don't know for sure on the treatment of the sibling. That hasn't been

confirmed. Most likely happened at the same time. The cause of delay in treatment was based on a lot of family concerns. There was a language barrier. There was also a request for a second opinion. The family was very concerned what was happening. That also caused some of the delay in the two-year-old sibling to not necessarily seek further diagnostic testing. All right. Next slide.

Our second confirmed case, the specimen was received on day two of life. The positive results was on day four of life. They found that the baby had four copies of SMN2 clinically and the treatment was scheduled on day 11th of life. It hasn't officially been confirmed but we don't have any concerns that the family didn't get testing on that.

Now, this is the real great story of how quickly the turnaround time could be for testing and getting the kid in and getting treatment as soon as possible. Next slide.

That's our quick summary. There's my contact information. There'll be questions at the end but if you have any questions on more details of our validation or the specific assay that we're doing, you can feel free to call me, email me and I can send that onto our other scientist who run it every day. Thank you.

Patricia Hunt: Thank you, Carrie. Our last portion here will be on the overview of second tier testing methods. Mei Baker will be the presenter. Dr. Mei Baker's an associate professor in the Department of Pediatrics and co-director of the Newborn Screening Laboratory at the University of Wisconsin School of Medicine and Public Health. Thank you.

Star seven, Mei.

Dr. Mei Baker: Hello?

Patricia Hunt: We can hear you.

Dr. Mei Baker: Great. Thank you for the opportunity from APHL QC committee. I feel like I've been keeping getting demoted. You just introduced myself as associate professor but it's ... Just kidding.

I want to start with, this is table people have to see over times and have come to SMA and based on the age of onset and the motor ability and then life expectancy, we have subtype like I, II, III, IV. You haven't heard a lot so I'm not going to go detailed. I do adding on the one column in terms of SMN2 copies because in large, the relationship determining SMA severity and SMA II copy numbers are inverse. In other words, if have more SMA II copies, your phenotype could be less severe. Next slide, please.

This is recently the publication and the people may see that and they saw that from Dr. Jennifer Kwon's presentation. This is the group from Spain and have a

625 patient. For the interest from time, I'm not going to detail so you can tell when SMN1, zero, you have more SMN2 copies and the symptoms is less severe. Next slide, please.

I think we talk so much about SMN1, SMN2, and I would like to have the opportunity demonstrate what's the relationship. From this flow chart of process, when the, RNA truncates from DNA and have to go through the color splicing process to generate the messenger RNA so technically, in trying to, it comes out then have a coding area linked together. We already have heard SMN1, SMN2, the critical difference is in exon have the one nucleotide difference, C to T difference.

In the SMA I, you have a C has been considered of exonic splice, the splice sequence, which is enhanced the four lines messenger RNA but if we do change it SMN2, you have a T and potentially can cause a skipping so you will miss exon 7. You have a truncated a protein in the end and the functions are quite different. So, it's why it's so important. When we have one or two SMN1, how many SMN2 is, it doesn't matter but the one you do not have SMN1 and SMN2 copies can be important. To my knowledge, that's great is anywhere from zero to eight. Next slide, please.

Come to the assay and newborn screening related. Here is the demonstrate, actually that's Francis Lee, Dr. Lee has given you very comprehensive overview in terms of available method of newborn screening for SMA. This I demonstrated here, it's our assay based on the prototype PerkinElmer assay and which he is similar to CDC version two. You can tell in this assay, actually the primer set actually will amplify both SMN1, SMN2 but that the probe will separate SMN1, SMN2. Personally, I like this design for the following two reasons. One is if you have this probe, SMN2 probe can more specific for SMN2 and SMN1 probe in there that can block SMN1. This way, you have more specific amplification. Second one give us opportunity to also assess SMN2 copies. That's the rationale we want this direction. Next slide, please.

Another way to do SMN2 copy numbers is use the droplet digital PCR. I think I should mention another method called MLPA and Dr. Lee also mentioned that but I think that time is before droplet digital PCR available. In our hands and droplet digital PCR, the performance is very simple just so you do any real-time PCR except have a one additional step is generate a droplet. The purpose is that you create individual one molecule applications. The software will count each individually and separate them in this partial the category. The blue dots on the left top part is either SMN1, SMN2. You can choose to do which one which is commercial available to buy it, have it as a kit. Orange on your right top is that droplet content the both the SMN1 or 2 in combination with the reference gene, which is green on the bottom part and the bottom left side is the empty droplet.

This is sorting out and software will counting that and you can assess the ratio. The second part, the lower part is the indicator, is they will access the copy numbers. This is all automatically. When you click certain parts, they will conflict. You can choose, ask either way, either because all compilations are based your SMN1 or SMN2 ratio with your reference gene because we know reference gene is two copies, so based on that will give you this. Next slide, please.

This our we have a group samples, we try to compare with real-time PCR and droplet digital PCR. The interesting thing is, you can tell this is all true patient samples. They have a clinical diagnosis type and also we ask tell us clinical diagnosis give what is SMA II copy numbers. Unfortunately, copy sample they didn't provide. I want to join your attention to this red one. Even though we have one case SMN type 2, clinically, and we was taught they have three copies and real-time assay will tell us it's four copies and a droplet digital PCR is three. If you want to say, "What's the component here," personally, I would say that's the match up clinical type better. That's indicate the droplet PCR even more accurate.

Case two, everything's concordant and case three, very interesting because the clinical samples that come back to us say this is a type two and you would expect that it's above the three copies but we were taught it was four copies. The real time also said it's four copies but our droplet digital PCR said it's three copies. It's more streamline with type two.

We understand largely they will have an inverse correlation but have the exception but also make me start thinking about maybe technology can play some role so we should keep this in mind. Of course, we need more data. I can skip to case four and five and six because this is pretty concordant and interesting case seven. The real time said that's more than four copies but digital PCR said this is three copies, also more correct fitting in the clinical type.

In our, after we see more data, see both real-time PCR and droplet digital PCR assessment, SMN2 copies, we decide we will go with droplet digital PCR. We have the machine. We didn't buy high throughput because we think we are not in need of high throughput so we can do it each time do eight samples. Next slide, please.

In general, as Dr. Lee mentioned, that we already have a fully validated good SMA screening assay and we are state advisor group already proved, move forward with the SMA screening. We just waiting for the logistical things to dump that laboratory is ready. In our screening protocol, we started with the real-time PCR, then indeed, we just like other, everybody else, we were only report SMN1 deal cases and all the new cases will undergo droplet digital PCR reported SMN2 copy numbers. Next one, please.

This is our kind of procedure and indeed, after we even, we report SMN2 copies but we will ask an independent specimen either in our laboratory or in the reference laboratory for reassess SMN1 and SMN2. SMN2 copies one or three will immediately refer for the treatment and therefore, SMN2 is four or more, actually we'll assess the symptoms. Without symptoms, there were clinical follow-up without immediately put under medication. Next one, please.

In summary, and we believe this is a technically feasible to incorporate SMA screening test into current, ongoing screening tests that use the multiplexing and everybody else will tell you how cheap you all be. Second one is it's feasible to avoid SMA carrier identification by only detecting SMN zero cases, because also newborn screener so we all know and other situation, we are not able to avoid it but SMA, we can.

The third one I want to emphasize, the previous presenter already said, newborn screening for SMA, we really is screening SMN zero so yes, other type mutations and we all have an limitation so our sensitivities will have to be very, very clear at the beginning in terms, if not 100%.

Also, I feel it's the benefit to including SMN2 copy numbers assessment in newborn screening for SMA protocol. We all know, in terms of SMA type IV and it's not very common from a clinical exhortation but my prediction is because of newborn screening identify SMN zero, we will see more SMA type IV. I think it's important we have commonly SMN2 copy numbers to help us to better consultation and counseling with the family. Next one, please.

I just want to say this is really a teamwork. We do have SMA work group. Dr. Meredith Schultz and Dr. Matthew Harmelick is our neuro/muscle doctors in the two locations so there were follow our patients and Audrey, actually is coming into the role because we are prepared to identified SMA type IV even "normal" because the one you have SMA, SMN2 copy five, six, seven potentially, they will never have a symptom. We want to be sure do something assessment with the family direction. Audrey is a very experienced in CF screening to assess the family reaction and psychosocial reaction, stuff like that. Anita is our coordinator. She keeps everybody on track.

Also, I want have special thank you to the people in the laboratory and Sean Mochal. He is very talented and knowledgeable and humble and does a lot of work with both SMA I and II assay. Mandie has working with Sean to do the SCID SMA I assay. Bethany had recently joined Sean to do the droplet digital PCR assay. Thank you.

Patricia Hunt: Thank you. Thank you to all of our speakers. Due to some technical difficulties, we're running a little bit behind so thank you, audience members.

We will hang on for a little bit longer. If you want to ask a question, you can press star seven to unmute and go ahead and ask a question or you can use the

chat box. I'm sure we'll try to, if you're unable to hang on, then you can contact the speakers directly with your questions or contact Funke at APHL. We'll try to get some responses. Please, if anyone is on the line, go ahead.

Funke: There is a question in the chat box. It says, "What is the status of SMA with respect to the HHS Secretary's response to the letter from the advisory committee? When do we expect a formal response? Is there a hard deadline?" This is open to any of our speakers.

Anne Comeau: I think the bottom line is we don't really know. Does anyone have a harder answer? Michele?

Funke: Yeah. I think this was touched a little bit in her slides. Unfortunately, she had to drop off but if there are any other questions directly towards her, we can follow up with her after the webinar.

I can move onto the next question. It says, "How soon should an affected baby be treated to avoid permanent damage?"

Dr. Mei Baker: We can give a try. This is Mei. Interestingly, this disorder is unlike other disorder and deficiency, whatever that can have a ... You know, get an enzyme for mom. This one neuron's died, were never coming back so the sooner the better.

To my knowledge, I would say within one month, because at a clinical trial, they used the six month old. It's just not ideal so you have to be SAP. Again, the principle is the neuron died. The neuron won't ever coming back so sooner than you will be protect more neuron to die but you think about it because you give treatment and take some time for them to become a fact. Other people, they have ...

Anne Comeau: Mmm (affirmative). This is Anne. I tend to agree that most of our clinicians have indicated that they call this a pediatric emergency. They want these babies seen as soon as possible. That said, I will also say that this group of clinicians is not as familiar with newborn screening processes. I think I'll speak for myself but I think I also heard it from Utah that when you begin this assay and you have what you think might possibly be a positive, you run with it because you don't want to, if a baby is already one week old. Then, it's going to take some time to get the confirmation testing done before they can do the treatment, it's unnerving. What is not unnerving is that I think all of these assays are really beautiful assays but until you've run them a little while, you have to have the confidence to know what you think is a true positive and not.

Francis Lee: This is Francis Lee. I've been talking to the neuromuscular specialist. How fast you have to treat really depending on how severe, how fast the progression of disease is, which obviously also depending on a panel of factors, where there's going to be Type I, Type II, or Type III. The biggest worry is, of course, Type I.

Type I, the nerves stop buying perinatally and by three months, you can have a really severe deficit. By six months, you can have lost 90% of your nerves.

It is scary but the thing is, with this newborn screening, you are going to treat them during the asymptomatic stage. We can only guess, predate from their SMN2 copy number but there are other modifiers. Genes, for example, SMN2 has a very and that can decrease the severity of this. There are a lot of things that we won't get because you don't wait till the symptom appears. It is a little bit concerning to me because we really base your treatment, your management based on the genotypes with no help from phenotypes at all.

Actually I just came back from the Cure SMA meeting. They will be reporting a survey on a panel of 15 neuromuscular experts. They have decided, if you have one copy, two copy, or three copies of SMN2, you go ahead and treat right away.

When you have four copies, the panelists advise that 50% of them say, "Go ahead and treat," and 50% of them say, "Well, you might want to wait and watch." Then, attach for all the modifying genes. I think the consensus, if you worry about type of SMA, you go ahead and treat as soon as you can.

Patricia Hunt: Thank you all for the responses. Funke, do we have time for one more question?

Funke: Currently, there are no more questions in the chat box. If anyone has a question, they can press star seven to unmute themselves.

Patricia Hunt: Thank you everyone for hanging on. I would like to thank all of the presenters. Thank you very much for sharing your experiences.

The SMA webinar will be archived and recorded and will be available on the AHPL website within a week and part one that has some clinical information as well is already posted if you want to take a look at that. Thank you very much and deep apologies, Mei. We obviously have to update your bio.

Dr. Mei Baker: Thank you. I was just kidding but ...

Patricia Hunt: Promote you twice. Thank you very much everyone. On behalf of the APHL newborn screening QAQC committee and special thanks to all of the APHL folks that helped put this all together. Thank you very much.

Funke: Thank you all.