NewSTEPs
A Program of the Association of Public Health Laboratories™

Severe Combined Immunodeficiency (SCID) Webinar

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Please direct all comments/questions pertaining to this webinar to Ruthanne Salsbury at Ruthanne.salsbury@aphl.org or 240-485-2767

Ruthanne Salsbury: Okay, great. Good afternoon everyone, this is Ruthanne Salsbury on the line, and on behalf of new steps and the newborn screening translational research network, we would like to welcome you to this month's SCID national webinar. We're so glad that many of you were able to join us, I know I had a couple of emails from some new folks for this month so welcome. For those of you that are joining for your first time this month, the purpose of these calls is to address legislative and regulatory challenges, laboratory methodologies and techniques and follow up activities relating to SCID. I'm now going to review the agenda for this month.

We will begin with a SCID screening, Newborn screening map. Then we will have some highlights from some SCID [inaudible 00:00:54]. Regarding the SCID implementation experiences. Just to give you some background, in 2014, APHL was awarded 4 million for a collaborative agreement from the health resources administration of the US Department of Health and Human Services, and under this cooperative agreement we've been supporting 11 newborn screening programs towards school implementation of SCID newborn screenings. Today, we are very delighted to have 3 of the programs that have already begun screenings, shared their experiences. It will be Maryland, Kentucky, and why, and then following their presentation, we will have [inaudible 00:01:35] share some more information about the following infants with low lymphocytes [inaudible 00:01:41] project.

We have a pretty packed schedule today, if you could just save your questions for the end of this call, that would be great. I have muted all the lines, so at that time, I will ask you press star 7 to ask a question. You are also welcome to ask questions throughout these presentations in the chat box on the bottom left hand corner of your screen. We are looking forward to pack the agenda today. I'm going to start now with the SCID newborn screening. Matt, as you will see, we have a couple of updates since our last call as of July 2016, we have Vermont, North Dakota, and Alaska now screening. That's really exciting. I encourage you to visit our newsteps.org / SCID website to get more information about state screening status. We look forward to having many more programs begin screening over this next year.

All right. With that, I'd like to hand it over to Dr. Fizza Gulamali-Majid who is the division chief, and Dr. Adam Coleman, who is laboratory supervisor of the division of

newborn screenings within the Maryland department of health and mental hygiene. If you all would just press star 7 when you're ready to present that would be great.

Adam Coleman: This is Adam Coleman, I'm not sure if Dr. [inaudible 00:03:32] is on the line. Maybe, is she? She's out working from home today. I don't hear her voice, so I'll just go ahead and get started. My name is Adam Coleman. I am the supervisor in molecular biology in Maryland newborn screening. Right now, that's only SCID, but hopefully in the future we'll have some more tests. Just to give you a little background, we have about 70,000 births per year, we're two sample states. I'd like to mostly give you some laboratory data we have. We started screening 6 months ago and we have just sort of drawn a line in the sand, and said okay 6 month data, let's collect them and go through them as thoroughly as we can. I don't have data such as follow up, like number of babies diagnosed or anything like that, but I do have access to laboratory data, so I want to show you some of the barriers we've hit, and hopefully folks can learn from it or maybe teach me how to get through them.

Just some background. We use [inaudible 00:04:47] to do our SCID tests. In this, we have a dry blood spot, and we wash them in solution 2 which is made by [inaudible 00:04:57]. Then the solution 2 is removed, and we add quantitative PCR master mix. We measure two things, TREC, T-cell receptor excision circles, which are a marker of T-cells, and RNHP, which is just an internal positive control. Basically we're looking to see if the babies have T-cells in their blood, detectable T-cells by looking at TREC. We use a CT based cut off, I don't have the time to go into exactly what that means, but what you need to know, is that a CT value, it's inversely proportional, so the higher CT value means less starting material, or a lower quality PCR reaction as a whole. The next slide, please. Okay, so as I said before, we're a two sample state. We decided that we were going to screen all the samples that come in, even subsequent samples that have a previous normal screen. We define in our lab newborn as collected less than or equal to 7 days of life, and then subsequent is after that.

In our lab, we call them newborns and subs. I'll probably slip into that slang, so I apologize for that, but that's just how I talk. We started live screening on April 1st, and so our 6th month-anniversary was October 1. There's a total number of screens, or the [inaudible 00:06:41] we've gone through. Next slide please. Okay. This is our algorithm, I'm putting it up mostly just for records so that folks can look back at it if they're interested. I don't want to go into the details of it. Basically, we do a single punch on every baby, and if there's something strange with that punch, if the RNHP or the TREC is bad, then we will do a duplicate re test. Then we have 3 total data points for each specimen, and then we make a call whether or not to refer them for further testing or get another sample or declare they're normal. This is a huge amount of data on one slide here, this is about I guess 75,000 QPCR reactions that are represented here.

This is the two barriers I want to talk about are related to this slide. This covers a whole 6 month period, [inaudible 00:07:49] it's hard to read, but it's a lot of data. We have in red the newborn specimens, and so I'm graphing there the daily medians, so all the babies receive that day what is the median TREC value. The same with the

subs, the subsequent specimens, and then I have a moving average there. As you can see, the data is interesting looking. A little too interesting looking and that's been a source of some trouble for us. We realized early on that the subsequent specimens had a different population distribution than the newborns, this was unexpected, at least to us. We hadn't seen it published elsewhere, but we found that the subsequent required their own distinct cut off values, different than the newborns because they just had different distributions.

We discovered that after the first couple of weeks of screening, so all along the left hand side there. Then, things got even more interesting. Can I have the next slide, please? After the first couple of weeks, you can see there all the way on the left hand side of the big chart that the newborns and subsequent samples had different medians, but they were at least parallel from day to day. Then around the middle of May, things started to get a little odd and as you can see, the newborns, the red, has been, there's fluctuations day to day. Over the whole 6 months, basically flat lined around 30 cycles is the median, and that hasn't changed. The subsequent specimens as you can see are up and they're down and they're up again, and this has been a real headache for us because nothing really has changed. The only thing that really changed in May was sort of the rising subsequent median, the only thing that really changed was my blood pressure. I'll pause there for laughter because you guys are all muted.

Okay, great. Go to the next slide please. The real problem with this shifting, as you can see here, the black line is the mean, the mean is the first, okay, let me back up. I chunked the data into 2,500 specimen chunks. Then have graphed them here in histogram format. The y-axis is the frequency of that CT value occurring. In the red you can see that over the couple of first months, the newborn specimens have basically the same distribution. Same width, and the same sort of outlier percentage. The red cut off you see there has been very effective for newborns. With subsequent samples, however, we've had this migration of the floating of the subsequent population. As you can see, if that whole bell curve of subsequent samples moves to the right, it pushes part of the bell curve, part of the very healthy baby bell curve, part of that bell curve goes over the abnormal line or cut off line.

After we thought we had fixed the sub problem by giving them their own separate cut off, in a couple weeks, we found we were again referring way too many subsequent samples that we were chopping off a portion of the healthy population and referring them for further testing, which we did not want to do. The cut offs were inappropriate. The problem is, the subsequent population is constantly on the move. This took us a little while to get our heads around it. Finally, we decided to go with a daily cut off system.

Can you go to the next slide, please? Basically what we had to do is add this extra step in, so after we do the initial screen for every subsequent specimen for that day, when we total up all that data, we collect the median, and then we set the two cut offs sort of in accordance with that median. Our sort of soft cut off, our re testing cut off is set at the median plus 3 cycles and then the final cut off, the hard cut off, is 3.32 cycles.

We came up with that looking at other states' algorithms, and also using the CDC calibrator material. We knew where the linear range or the assay was, and where we could set this reliably. This has added an extra number of steps for us each day to get the subsequent cut offs right, but we've really cut down on the number of presumably false positive ones. Now the cut offs are more appropriate per day. Next slide please.

The next problem has a little bit less satisfying a solution. At the beginning of August, we received our first shipment of solution 2, which is just the wash solution, and we did the new lot verification like you do. We could not get the stuff to pass. We couldn't get the new lot to look like the old lot, but the thing is, it was giving better results, I should say, lower CT value results than the old lot, go to the next slide please.

We couldn't get it to pass, but it turns out the new lot actually looked more like the old lot did at the beginning of the year, if that makes any sense. We're faced with an old lot that apparently had aged poorly. At least that's a theory. That it was giving different results than we had when we started, and the new lot that came in was giving very similar results to at the beginning. This was really surprising, because the new, or the old solution 2 had a couple years left before it was expired according to Kyogen.

It's not like solution 2 is all that fancy. It's a room temperature storage situation, if you just set it on the bench, you don't have to protect from light or anything crazy like that. We couldn't figure out how it is this was happening, but this is what happens. This is the way it looked. Can you go to the next slide, please? It affected different samples in different ways we have found. We haven't been able to do, like a very in depth analysis of this. This is something we are still very interested in and we are collecting data actively and hoping to publish something on all of this data actually.

I wanted to share it with you just in case it would help some of the labs. As you can see, the blue sample there, the sample in blue, had a real big shift as the old lot aged and then when we got the new lot in, it looked much more like the old lot had originally earlier in the year. Another reference sample we had is QC borderline, we call it. Didn't change very much from old lot to new lot. I can't speak to the why of why this reagent has failed us, has apparently failed us. I'm not sure if this is a one off event that we just had a lot that ages poorly. It's also possible. Kyogen doesn't manufacture this for this purpose, so it's very possible their expiration date and their quality control procedures don't really account for this kind of use. We're putting it to kind of different use than it was intended. It's possible their expiration date just might not be valid, that it may have a much earlier expiration date for doing the TREC assay and that's something we are investigating further. Next slide please.

I don't have an answer to the solution 2 stability issue. I have an N of 1, basically. I have an old lot and a new lot, and I see these big differences and I can make lots of pretty graphs and hand wave, but I can't really tell you what the difference is or really what to do about it. We're just going to try to keep a close eye on our solution 2 and

keep you updated. Hopefully the folks at the CDC can address this more directly if this is an issue. Maybe other labs haven't seen the kind of subsequent wandering that I've seen, because maybe our solution 2 is unique and aging poorly. Maybe that was part of the problem with that.

As you can remember, the graph, the new lot has very parallel lines between the subsequent and the newborn examples. Kind of like it did at the beginning, perhaps that's why I see that and know why the lab has reported that. That's another possibility. Next slide please. Basically, the lesson learned basically on the lab side, is that we need to keep really continuous data analyses happening. We've been trying to keep that up, I'd like to do a better job of that in the future, because science can go sideways on you sometimes and you need to be ready to protect the babies against that so you don't have a bucnh of freaked out parents or any missed babies. It's important to keep an eye, especially as we are currently starting and a couple other states are starting soon. You ought to really put some good quality control monitoring in place and keep a good eye on it, because ours has been kind of a tough nut to crack, it's been a wild ride. Next slide please.

I'd like to thank the APHL, new steps for all the money and all the help. They've been critical in getting this all started. We have a new member in our lab, [inaudible 00:19:08], who's a great hard worker and a huge help to us. I'd like to thank the folks at the CDC who are always around for advice. Our [inaudible 00:19:18] advisory committee, which is made up of doctors and neurologists in the area. The director of laboratory's administration, Dr. Meyers. I also like to thank Dr. [inaudible 00:19:27], the division chief because she's a great boss and that's all I have. I'd be happy to take any questions now or later, my email is at the start of the slides. Thank you.

Ruthanne Salsbury: Awesome, thank you so much Adam, it's really remarkable to see all the hard work and dedication your team has set forth. We have SCID forward in your state so we thank you so much for sharing your SCID experiences and sharing these awesome graphs with us. I am going to ask we save questions for the end of our webinar today, but if you have a question you'd like to ask, you're welcome to type it in the bottom left corner of the screen and we can ask those to Adam at the end of this webinar. I'd now like to move forward and introduce [inaudible 00:20:15] who is the laboratory scientist supervisor for the newborn screening department at the Kentucky division of laboratory services. Darrin, if you could press star 7 when you're ready that would be great.

Darrin Sevier: Can you hear me?

Ruthanne Salsbury: Yes we can hear you.

Darrin Sevier: Okay. Good afternoon everybody, and I'd like to turn it over to how we went about implementing screening for SCID in Kentucky. Next slide. To give you a little bit of background our law is written so we can perform any test that's on the recommendation from the American, this says American College of Medical Genetics,

that's the way the law was written even though now it's the secretary's advisory committee, but it's the same difference. The one issue we do have though is the law does not provide any funding so even though we have the ability to test per the law, we don't always have the funds available.

That requires revision of the Kentucky administrative regulations, it then has to be reviewed by the legislature before it can go into effect. We screen, our normal, we are a one screen state, and we have a birth rate of about 55,000 babies per year. This is our timeline of how we implemented SCID, so basically I know the ASMG, or the [inaudible 00:21:46], added SCID in 2010 [inaudible 00:21:50]. We of course didn't have the funds, but in 2013, we did decide to go ahead and start working on implementation because within 3 years and we knew we needed to start sooner rather than later. Then in December, we began work on the fee increase, which of course was a total reroute of the administrative regulations, because not only do we have to ask for more money in the fee, but we also have to spell out all the testing we perform there, and any further follow up testing that has to be performed also has to be added in to that administrative regulations. That's not necessarily a small process.

Which that took awhile, and then in August 2014, we went ahead and ordered the thermocyclers, and then attended training in CC in September 2014 because we decided based on our birth rate of 55,000, we could do the CDC and [inaudible 00:22:47] method fairly easily with little [inaudible 00:22:49] and no need for robotics. Then in November 2014 we applied for and received the APHL SCID grant, next slide. Then we began testing, doing the validation in January 2015. We took about 6 months to work out all the kinks, decide how we're going to do things. Talk about the follow up procedure and work on algorithms and that sort of thing.

We looked through, we consulted with a couple different states, and we met with our pediatric immunologist and our follow up personnel to kind of work out all the kinks. In the meantime while this was happening in April of 2015, our fee increase did become effective, I'll talk about that a little bit later. Then August 3rd 2015, we began our pilot testing. We called it pilot testing, it really was basically just going into the new screening testing, but we didn't have a way of reporting the results at that point, and I'll talk about that a little bit later as well.

I also noticed we do have a typo in the slide. April 6th, 2016 was our official [inaudible 00:24:12], not 2015. We were still validating in 2015. As I said, we applied for the SCID grant, the funding period for us was October 1st, 2014 through September of 2016. We received approximately 150,000 each year for two years, and we used these funds for salaries, travel to the SCID meetings, supplies and reagents, the big one was LSD interface and maintenance. Adding those to our lymph system. We also used it for office supplies and printing supplies, other things we would need because as I said, at this point we had already purchased the thermocyclers, so that expense had already been made. Next slide.

We also in the middle of this, we had scheduled a map visit. This was mostly for CFDNA at the time, but we also knew that since we were going ahead with SCID, it

would be a good idea to bring them in and see if they had any input into things we might want to do differently, or work flow set ups, as you may or may not know the map visit is like, molecular assessment program. Offered in conjunction with CDC and APHL. They come in they review work flows, [inaudible 00:25:36], they will look at things. They were here 2 days, I think. Then you had personnel from CDC and personnel from APHL, and personnel from other state labs. At the end of this, they provide a report that has recommendations for work flow improvement. Ways that we could beef up validations. That sort of thing. Next slide.

I mentioned we had to increase our fee. We did increase our fee back in 2005, to account for the expanded newborn screening program. Had not been increased since 2005, so we had to increase it to 99 dollars. This included, we had been operating in the red for a while so this was not just for SCID itself, but we did include fees for SCID testing. Enter the increase. We bumped it to 99 dollars. As addendum to this, we had a law passed that we had to perform [inaudible 00:26:36] testing. [inaudible 00:26:38] were added to the rush, so we had to begin testing for that, and we had to increase the fee again to pay for testing for those 3 LSD disorders.

That was the original fee increase was effective in April 2015, then in April 2016 we had a new fee increase just a year later. Next slide. What lessons did we learn? Our original plan was to start with 2 thermocyclers. This was going to be used for validation and at the beginning we thought we could get by with 2 thermocyclers, but we knew we might need a third one later. However, when we purchased the thermocouples, [inaudible 00:27:26] discontinued them shortly afterwards, so now we're unable to add a third thermocycler, which on most days it's not a problem, but when we have heavy days or if one of the instruments goes out for some reason, then we don't have enough thermocycler capacity to complete the testing in one day, so then we have to, it spills over into a following day. There's worse potential for a bigger turnaround delay if we have a longer downtime than one or two days, because with one thermocycler, we can pretty much only run two to three plates a day. Next slide. Here's our algorithm. It's quite a bit more complex than what Maryland just showed, and I don't expect you to read through this right now, you can have it for later.

We started out with our pediatric immunologist and he wanted to look at premature babies versus normal birth weight babies, we basically are basing this on gestational age, but if the gestational age is less than 37 weeks, which we consider premature then we also start looking at birth weight, transfusion status. Mostly this is the trying limit, the number of specimens that we refer is high risk, because we realize in premature babies, you're going to get lower TREC values just as a matter of course. You guys can look at this as you want to, and I'll talk about it a little bit more in a minute, so next slide. As I said, this was developed in consultation with our pediatric immunologist.

He had a lot of good insight into how he thought things should be followed up. Based on some of his literature reading and some of his experience working with SCID patients at other facilities, also we consulted with other states that were already

performing SCID. New Jersey and Ohio were very good at helping us during our validation and afterwards with issues we were having. The biggest issue we had was implementation of the logic into our laboratory management system, basically because the algorithm was very complex, and so it took a while to work through all the permutations to get all of the result codes available in the limb system to be able to report it out. It actually is working very nicely now, it just took some time, that's why our pilot ran from August to April, and it was basically just because it took that long to get the reporting piece into our limb system. We still are working on how we handle repeat subsequent specimens because a lot of times you'll have normal on the initial but then you'll get an abnormal on the repeat specimen and there's been some debate as to how we handle those.

Do we refer those out for further testing, do we just ignore that result? Do we modify it in some way? We are operating based on just the gestational age, so if the baby comes in, we will look at what the gestational age is on the repeat specimen, and then go by our algorithm that is showed. However, sometimes that's difficult, because on subsequent specimens, we don't always get as good as information as we do on the initial specimen. We've also had issues with doctors that are confused by getting multiple moderate risk specimens. As our algorithm stands now, if it's moderate risk but it's a premature baby, we will ask for a second specimen in two weeks. If the baby comes in again and we have another moderate risk result, but the gestational age is still less than 37 weeks, we will ask for another repeat specimen. We've kind of trained our PCP's with some of our other disorders that if we have two borderlines in a row, that becomes a referral and so we have had doctors that have called and said why is this not being referred for follow up testing? There's an education issue, there as well. Just trying to break the habits of some of these doctors. Next slide.

A little bit about our experience so far. We have screened, since the pilot, 56,707 babies, and I have to note that that is from pretty late August 2016, so it's about 6 weeks behind. Since we went live in April, we've screened 20,385 babies. Next slide. Out of the percentage of the total screened, 83% of those babies were full term based on gestational age, and 70% were less than 37 weeks gestation. Then if you look at birth weight we had 89% who were normal birth weight, and 11% that were less than 2,500 grams, which we consider low birth weight. As far as percent of abnormals, so these were the specimens that were not, had a elevated TREC CT value. In the premature specimens, obviously we had 81% that were premature as would be expected, and only 19% of the full term babies had abnormal results, or 19% of the abnormal results were from full term babies. When looking at birth weight, was a little bit more even. You had 67% of the abnormals were low birth weight and 33% were normal birth weight. Next slide.

If we get an initial moderate risk and we ask for a second specimen, what did those results turn out to be? Granted this is only in a 30 abnormal results that were coming back where we get repeat specimens on, but as you can see, the majority of them confirmed that as abnormal when we got a second specimen in. A 54%. 20% were still moderate risk. We had 3% that then transitioned to high risk, which for us, high risk means referral to a pediatric immunologist. We had 10% that came up as

inconclusive, so that would be abnormal TREC and abnormal RNHP, and then 13% as of the time the slide was made we had not received repeat specimens on. Next slide.

That gives us an intimate rate of 1 out of 28,354, and that's based on 2 positive cases out of the total we've screened since August 3rd of 2015. Next slide. Our two cases we've identified, our first case was an AD8 efficiency, and this was found I believe 2 weeks after starting pilot testing. The gestation has underwent gene therapy, several months ago and is doing well. Our second patient we identified a couple months ago, and that was a cartilage hair hypoplasia. The patient had no significant infections to date, and scheduled to have a bone marrow transplant when the baby is 6 months old, because the baby was pre term, they wanted to wait until the organs matured before they did the bone marrow transplant. Next slide.

That's all I have. I'd like to thank our pediatric immunologist Dr. Lee and our follow up program, and our university centers that take care of our referral babies. In addition to the rest of the staff here at the division of laboratory services. Thanks.

Ruthanne Salsbury: Awesome. Thank you so much Darren, it was really wonderful hearing your program's experience, and for those of you that might have specific questions for the Kentucky program, I encourage you to reach out to them directly. You're also welcome to send emails to me and I'm happy to connect you with them. These webinars are recorded so all these slides will be available online. I'd now like to introduce Sylvia Mann, who's a supervisor of the genomic section within the Hawaii Department of Health to share Hawaii's good newborn screening experience.

Sylvia Mann: Aloha everyone.

Ruthanne Salsbury: Awesome.

Sylvia Mann: Ours is not laboratory, ours is follow up, our SCID project, and so after listening to the two lab talks, I definitely want to start off by thanking our contracted Oregon State public health lab for taking care of all these lab problems for us so that we don't have to deal with them. Our project is on following up SCID using a collaborative partnership. Next slide. The background of our project is that we do not have any resident pediatric immunologists that work with immuno-deficiencies in Hawaii. We have pediatric infectious disease docs and bone marrow transplant specialists who work at our children's hospital, our only [inaudible 00:36:55] children's hospital in the state.

In order to actually do comprehensive follow up, we needed to develop a collaborative partnership with a center outside of Hawaii, and luckily our bone marrow transplant specialist in one of our pediatric infectious disease people now, were trained at the UCLA [inaudible 00:37:19] Children's Hospital, so they were able to contact them and they were perfectly willing to collaborate with our Hawaii program. Next slide. As part of the project, we of course had to figure out logistics because we're dealing with UCLA specialists, working with specialists in Hawaii.

We need to schedule regular video conference meetings and to schedule our regular video conference meetings with so many specialists and the health department people, our new bunch of [inaudible 00:38:00] program people and our genetic specialists. All at the same time can be challenging, of course, especially with the fact we have a 3 hour time zone difference, part of the year, and then a two hour time zone difference the other part of the year because Hawaii does not switch to Daylight Savings time. Also, everyone's very very busy schedules. Our communication on a regular basis about the newborns were definitely influenced by distance in our time zones.

We also were very happy to know both of those medical centers shared the same Epic EMR, but even though both IT specialists and O centers have been working on the system and we've been testing being able to share and send messages. We still have problems with that and it's very difficult, because there's a lot of lab results that have to be transmitted back and forth from Hawaii over to UCLA, and so the only other alternative is to fax all the results which is not the best alternative. I think the last meeting we had, we might have come up with the reason why we cannot successfully have the sharing even though we can show the messages go back and forth. One of the things one of the doctors who recently did move from California realized was that when he worked in California in order for both sides to see the same record the UCLA site actually has to create a record for that patient even though the patient has never been seen or registered at UCLA so that record has to exist in their EMR in order for the UCLA docs to actually receive the messages in the Epic system that are being sent by the Hawaii Epic system.

We're testing that our right now, so we're hoping that's the solution to it all. Next slide. Challenges for our follow up. We've been screening since March of 2015, and we have had no true SCID cases found. We've had several newborns detected with these unspecified immuno-deficiencies, and our main question now is well, these aren't really the babies we said we were going to be looking for, and they have very weird results and also it's interesting things as a practitioner, it's interesting to all of us, but as a newborn screening program, how should we follow these newborns? It isn't one of the disorders we have on our newborn screening panel, and they're so kind of strange that they're not even secondary targets. Definitely. The other part is our collaboration was developed to look after SCID cases, but because there are these strange cases, our specialists in Hawaii need more direction from the specialists in UCLA.

How does it work when you have a collaboration and then you end up getting cases that are actually outside of what you originally agreed you'd be working on? Then, as a newborn screening program, how long do we follow these cases to see what's going on? We know it would probably be useful to have some kind of national database where people could put in these cases that are found so that we could look up when we do have a strange case and figure out what was the outcome of this case that we might be able to have some idea, whether or not we have to do anything more intense or this is something we should just wait and see because the babies' immune

system will start to kick in at some time. We are having trouble figuring out once we get a whole lot of these babies, how long do we keep following them with our newborn screening program? Next slide.

Our opportunities. This has been an extremely great learning experience for all of us. The collaboration has been wonderful. Our UCLA collaborators are extremely generous and learned more about immunology than I've ever wanted to learn in my entire life. We've also been able to tap into our UCLA specialists to help provide continuing education for our pediatric providers. We had a wonderful half day continuing ed session where we brought the UCLA people over for a meeting, plus they gave the half day continuing ed session and we have all the talks recorded, so we will be providing links to Ruth and so that any other states that would like to see the presentations that the UCLA specialists. You're welcome to look at them. They were wonderful presentations, very very easy to understand about SCID and follow up and treatment and then the transplanting, because all of them teach at UCLA, so they did very well at presenting the information.

We also, because they live in the world of immunology and EMOC, they really have the ability to share resources with us. What kind of research studies are available. All of our babies that had the strange results had the regular SCID panel, mutation panel ordered, and all of them came out without any mutations detected on a regular SCID panel. UCLA was able to help direct us to Dr. Jennifer Puck at UCSF to try to see if our patients qualified for her funded study to do [inaudible 00:44:33] sequencing. Our patients qualified, my only caveat to that is that we have had trouble enrolling the patients because the way the research protocol is is that her staff, genetic counselor, has to do the informed consent with the families, and that's been very difficult to arrange that with our families that are looking after children, especially health needs. Trying to arrange with the busy schedule of the provider at UCSF.

We also are able to compare our laboratory experiences. We had a very horrible time trying to get timely gene mutation panel results back. In a reasonable time period, and so between the UCLA center and our center, we've been looking at the different laboratory options for the mutation panels, and been able to share what the experience is with the different panels and the costs. Also, the return of results time, so that's been very helpful too. We developed a chart of all the different labs that do see regular gene mutation panels, so I can share that with Ruth Ann, so that anyone else that's looking at the different types of labs that might provide this and what the turnaround time is and the costs to just look at the chart because we just recently did it, updated it.

We also get to learn about continuing education opportunities from our UCLA partners. Whatever conferences that might be interesting or meetings, we were able to send our Hawaii team over to a conference in California that our UCLA collaborators identified, and when they came back they said it was extremely useful and very very good to help move forward in doing what we do. Next slide. I just want to thank, of course, Gwen Palmer who's our newborn screening program coordinator, she's the one that does all the day to day work, and Jen [inaudible 00:46:46], who's

our genetic counselor assigned to this project. Then of course our [inaudible 00:46:51] medical center specialists.

They're the ones in Hawaii who work hard to keep our kids healthy. Then, of course our UCLA Mattel Children's Hospital specialists who are nice enough to work with us. Then of course [inaudible 00:47:06], and then new steps for providing the opportunity for us to get funding to help us develop this collaborative relationship. I think the good part is that [inaudible 00:47:17] Medical Center has seen based on our pilot collaboration, how useful this collaboration is, and when our [inaudible 00:47:25] funding, through New Steps, runs out, then they have told me, they're willing to take on the support and the sustainability of this collaboration because they have found it very useful for the specialists at Kapi'Olani. Thank you.

Ruthanne Salsbury: Thank you so much, Sylvia. It's amazing to see all of your program's hard work and I'm really looking forward to sharing the resources you mentioned with the broader newborn screening community. I'm going to quickly transition to our last speaker today, and I ask for those who have any questions to just stay on the line perhaps a little after the top of the hour, we want to make sure we're able to address your questions. With that, I am very pleased to introduce Kara Calder, who's the director of the UCID Net research consortium for the last 3 years and she'll be sharing some more information about the bill project.

Tara Caulder: [crosstalk 00:48:20].

Ruthanne Salsbury: Perfect, we can hear you.

Tara Caulder: Thank you Ruth Ann, and thank you to our last speaker, Sylvia as well because that does tie really nicely into my presentation today. I'm going to be talking to you about the following incidents with low lymphocytes project, or as we affectionately call it here, Phil. A little background. More and more infants are being recognized to have low lymphocytes early in life and as we know, it's often a result of newborn screening for SCID. [inaudible 00:48:54]. In addition to SCID and leaky SCID which are the primary target conditions for TREC newborn screening, we're finding other conditions with low TREC and T-cells as well. Phil was launched in an effort to capture the spectrum of infants who were born with low lymphocytes and record how these infants were managed, and we do that through special reporting forms in the USID net national registry for patients with primary immune deficiencies, so that resource exists. The hope is that we'll learn the course and outcomes for these babies. For the first 18 months or so of their life. Next slide please.

I'm going to skip through this slide and the next slide and the next slide. These were all just here to convince you that newborn screening of SCID and its diagnosis has very much increased over time, so we all know that but since we're running low on time, let's go to the next slide please Ruth Ann. We all on this column know that the SCID diagnosis thanks to newborn screening and TREC test has increased, and we want to enroll those babies in Phil so we can follow that natural history in the course of their

treatment and how are we going to do that, and I've outlined it here. Since these children are going to be tracked through the USID net clinical research registry, enrolling into the Phil program requires patient consent, or in this case, parental consent for the babies, and we have a pathway has two possible options that I've outlined here. First, the provider identifies a baby who qualifies for this program, and the parent wants to enroll them, okay? If that parent is at an already USID net enrolling center.

We have about 40 across the US, so if that's the case, then they go through the guest pathway, and then that provider would just use their existing RIB approved consent form for the USID net registry, and they can sign the babies or the parents up there. Then, the local studies staff, I think one of the problems Sylvia was talking about, but the local study staff there, at that enrolling institution can enter the Phil data in our online forms for the 6, 12, and 18 month time points. If that provider is not at an enrolling center, and there's only 40 so it could be. Then we go the No pathway, and the interesting thing is that we have an online electronic consent form that's IRB approved that the parents can sign themselves on our Phil website. At the end of this, I'll share the link with you for that.

In that case, then the USID net staff would take over at that point and obtain the relevant medical information at 6, 12, and 18 months from the original provider. Then the staff here would take care of entering that data for each time point. Next slide please. You can see the study is designed to be longitudinal, and the data we're collecting is going to cover at least, right, 3 time periods. Birth to six months, which is our initial evaluation, and then the 6 to 12 month period where hopefully we gain some genetic mutation information, or even a definitive diagnosis in some cases. Then the one to two years of age follow up, which we would see a final diagnosis and maybe even some outcome information there. It is flexible, so if there are relevant, there is relevant information you would like to enter past that time period then we can do that as well too. The great thing about this is that all this data flows directly into the USID net national registry. It's stored on secure servers and then our staff here can easily access that for analysis. Next slide please.

One of the other unique features of the Phil program, is the available diagnosis categories, and again these diagnoses might evolve in one patient over the 3 time periods I discussed. Providers are asked, or the study staff is asked to give the best diagnosis at the time, at that data point entry time. The options they're given are based on the 2014 [inaudible 00:53:32] report. They can either be typical SCID, leaky SCID, [inaudible 00:53:37]. We also have the non-SCID T-cell [inaudible 00:53:40] categories as well, so there might be a syndrome of low T-cells like [inaudible 00:53:46].

Possibly a problem that's secondary to an ongoing disease process but with no intrinsic defect, something like a cardiac anomaly or pre-temper. Then there are the idiopathic low T-cell numbers as well, and they may resolve over time, with or without an established diagnosis, but if an etiology is established, then the patient will be moved into the appropriate category. That's the fluidity I talked about at first,

so maybe in an initial diagnosis, a baby might be, it might have idiopathic low T-cell, but by a 2 year follow up, they might have been diagnosed with an actual defect. Next slide please.

I'm going to skip through these kind of quickly as well since we're running out of time. This is just an example of kind of what it looks like, what our online forms look like. They're designed to be really streamlined and user friendly, which if anybody on the call has any experience, thank you, yes you can keep going. With the USID net registry, it just looks very different, you can stop there. Thank you.

That just shows you what it looks like on mine when you're entering that data, it's very user friendly, but again, all this data is combined with the USID net registry, the existing data that's already there. We collect data on all kinds of primary immuno-deficiencies, but for the purpose of this talk, we're just going to focus on the SCID groups, which is second from the bottom there. We've developed 325 registrants in our registry already who have been diagnosed with SCID and if you can go to the next slide please. This is just a breakdown of that 325 and actually it's combined and severe combined immuno-deficiencies to be gathered to bring that up. This is a breakdown of all the specific diagnoses that are contained within that number. If you go to the next slide, so what kind of data do we collect?

I won't go word for word but this gives you an idea of the types of data we collect in our registry. This is the information we collect on absolutely everybody regardless of their diagnosis, so you can see it's demographics, labs, the pretty robust history of infections and so forth. Next slide. Please. Thank you. This just breaks it up then we go further and do some disease specific questions as well where we're getting into the nitty-gritty of the criteria used to diagnose the diseases, through all it's molecular information, clinical features and treatment. What I wanted to point out here was that the [inaudible 00:56:25] project collects all of this information in one centralized location and then we can combine that information selected there with the national registry. We think this creates a robust tool for examining the course of these conditions over time. Next slide please.

This is just a cute baby, this is our Phil flyer, and it has our website on it right in the middle there. Www.usidnet.org/phil. You can visit that for more information and even if you have a parent of a patient who wants to sign up, they can go to that same link and they'll find the electronic consent form there. Next slide please. I did it in under 10 minutes, and I'd like to thank the clinical immunology society and the Jeffrey Model Foundation for their funding of this Phil specific work. The USID net registry is a U24 resource, and it's funded by the NIAID, and finally and very importantly, I'd like to thank the immune deficiency foundation who administers the USID net grant, and houses the national registry. Thank you very much.

Ruthanne Salsbury: Thank you so much, Tara. Thank you for the very quick but very comprehensive overview of the Phil project. I'd now like to open up the lines for questions, I know we had 4 different speakers today, so you're welcome to direct any questions to each of those speakers, if you feel more comfortable typing your message you can do that in

the bottom left corner of the screen, or you can press star 7 and your line will be unmuted.

Richard: Hi, this is Richard [inaudible 00:58:28] from Virginia. I have a question for Adam. Can you hear me?

Ruthanne Salsbury: Yes. We can hear you.

Richard: Adam, I'm not sure if I may have missed the early portion of your presentation, but just wanted to know if you had controlled for lot numbers for [inaudible 00:58:44] that was used for [inaudible 00:58:46] preparation as well as the TREC primers and probes, especially the TREC probe.

Adam Coleman: Can you hear me?

Richard: Yes.

Adam Coleman: Okay great. Hey, Rich. We have had 3 different lots of the master, the QPCR master mix, and the data has not a bump in the data, looks exactly the same. I don't have them noted there, but that's there. We are still on our same initial shipment of the primer probe mixes and yes. Those also have not changed.

Richard: Okay. Thank you.

Heather: Hello, this is Heather from [inaudible 00:59:32], can you hear me?

Adam Coleman: Yeah.

Heather: Okay hi. Another question for you, Adam. Is it Adam?

Adam Coleman: Yes.

Heather: Okay. As far as your observation with solution 2 that is not new news, actually. That is something, I hope you've spoken to the CDC because I shared this type of information with the CDC as well as Kyogen, probably as far back as 4 years ago. Have you had a chance to talk to Francis at all about those?

Adam: This particular issue, no, I don't think I ever gave Francis a call about it.

Heather: Oh, okay. I can tell you, you and I could talk probably forever about this, but this is something I had spoken to Kyogen about, and had given them a whole lot of data, like what you're showing, and in addition to that, and I also had elucidated from everything, it wasn't a primer probe master mix, it was definitely solution 2, and

again, this was probably in 2012. I even went as far as to test the pH of the solution because when we were doing in the very beginning, when we started SCID testing, our version 1.0 I'll say, we were doing a two wash, solution 1, and solution 2, and it was eventually in 2012, when I started just using solution 2, and so anyway, one of the ways we would differentiate between solution 1 and 2 is the QA measure for the technicians would be to use pH strips so they wouldn't mix them up.

Anyway, with that being said, I knew solution 2 had a high pH, and so I was like, I wonder if the pH is affecting the extraction efficiency, because when I saw this difference between lot to lot and I also saw that we see the same thing with say, for example, if you have a bottle you have open and it's been opened for a long time, say a couple weeks. Say you keep opening it and you keep closing it and et cetera, you keep using it. It will be worse, you'll actually ...

Adam: Yeah I have to say in my observation, true.

Heather: As a matter of fact, it is so predictable that when I start having more repeats, I have a soft cut off, and then I have the cut off for when I report things out, and the technicians even know, when they start getting down to the last of their bottle, they'll start realizing they're going to have more reports, because of the trend of you know, just the way, the animal, just the way the solution works.

Adam: Yeah yeah.

Heather: It has to do with pH. I had shared all of this information with the CDC, also with Kyogen. I was like hey, one particular lie I had specifically was really, really, really bad. It was so bad I couldn't use it. Anyway, they pretty much ignored me, really, when it's all said and done. Even though I had tons and tons of data, I had tons of you know, evidence, I had pH evidence, I had everything. They still more or less just thought I was crazy. That's sort of a phenomenon that we have been, really it doesn't affect how many we report out. It does affect, we do have a high [inaudible 01:03:35]. We go through one bottle a week, so I can usually see the trends and see we're going to have some more repeats towards the end of the week or whatever, but I haven't seen an increase because of the way our cut offs are set up. An increase in what I'm reporting out, to follow up.

You know, that's the first thing I thought when I saw your slide, was like oh I wonder if that's a solution 2 issue.

Adam: Do you have like, how often do you order it now?

Heather: I do still order in bulk once a year, however, I do ask to sort of pre test, lot numbers. Just so I can make sure it's not going to be a bum lot number like the one I had gotten a few years ago that was dreadfully awful. It's like the minor variations I can handle, the major fluctuation which I saw in this particularly horrible lot number, I couldn't use it. I do pre test the lot before I order a big shipment and then I order for the entire year.

Ruthanne Salsbury: Thank you, and I am so sorry to cut this conversation off, I just want to make sure that we get to one question that has been posed in the chat box, but I'm happy to connect you all after this call so that you can continue to discuss this. We do have a question from Louisiana. They've asked if anyone is getting Medicaid reimbursement and which CBT code is being used, and that is for any of the speakers.

Darrin Sevier: In Kentucky we don't bill insurance or Medicaid, we bill the provider, the submitter, so if they're getting reimbursed, I don't know anything about it.

Ruthanne Salsbury: Okay, well thank you so much Darren for sharing. Your stage experience and if you have any questions you'd like to pose to the broader newborn screening community, I definitely encourage you to ask these questions on our new steps [inaudible 01:06:15]. I'm happy to share more information about how to do so following this call and you'll be able to reach a broader audience. I did just receive a notification in the chat box that Maryland does not bill Medicaid. Now, given that we are at 7 past the hour, I'd like to just share the date for our next call. As we mentioned during our last call, this calls will now be taking place on a quarterly basis, so our next call is actually in January, so it's at January this same exact time. As always, please feel free to submit topics for discussion, and we welcome presentations from groups and efforts. Thank you all so much for your participation today, thank you to all four of our wonderful speakers for your very comprehensive and insightful presentations and we look forward to keeping in touch and sharing some of the resources you mentioned during this call. Thanks, everyone.

Jelili Ojodu: This will be archived too.

Ruthanne Salsbury: Yes, so this webinar will be archived by the end of this week. There is a link that was shared in the announcement of this webinar, thank you, have a great day everyone.