NewSTEPs
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Short Term Follow Up Technical Assistance Webinar
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Presentations:

- State Profile Colorado—Erica Wright, MS, CGC
- Short Term Follow Up theme—John Thompson, PhD
- Non-SCID T Cell Lymphopenias During the First Year of NBS in Washington State—Suzanne Skoda-Smith, MD
- Diagnosis and Short Term Follow-Up of Cases found by TREC NBS—Jennifer Puck, MD
- Winter Storm Knife—Beth Vogel, MS, CGC

Moderator: Thalia Wood, NewSTEPs

Please direct all comments/questions pertaining to this presentation to Thalia Wood at Thalia.wood@aphl.org or 240-485-2701.

Thalia Wood: If you have any suggestions for the future, make sure you contact me. I do send out a short survey after the call to get feedback and see what you like about the call or suggestions for future calls. I think we'll go ahead and get started. It is 3:02 and Erica, don't forget to do star seven and we'll go ahead and start with the Colorado profile for this month.

Erica Wright: Hi, all. Thalia, can you hear me okay?

Thalia Wood: I can, thank you.

Erica Wright: Okay, perfect. I'm going to talk a little bit about Colorado today, just give a quick state profile and I'm relatively new to the public health side of newborn screening, but I've been working with a sub-specialist for the last ten years.

When we all think of colorful Colorado, a few things that always come to our mind, we think of the beautiful mountain vistas, we sometimes think

of the buffaloes out on the prairies. Some may think of we were the first state to legalize marijuana if you were watching 60 Minutes last night and, of course we think of skiing and our poor Peyton Manning, who I don't think we'll be seeing play again in the near future.

Next slide, Thalia. For newborn screening, newborn screening's conducted through our public health department's laboratory, which is right here in the metro Denver area and we do screen for all the disorders on the RUS panel with the exception of CCHD.

I'll talk a little bit about that at the end, but our CCHD is primarily been delayed because of the issues we realized with being that a bulk of our birth population is at least born a mile above sea level, but we do even have hospitals that are closer to 8 and 9,000 feet above sea level, which very much causes issues with doing pulse ox at that level.

We began doing tandem mass spectrometry in 2006 and we also began doing SCID in 2012. We do screen not only Colorado, but we cover Wyoming and also some Native American reservations in the Arizona/New Mexico area.

When thinking about Colorado, we think of the Denver metro area and along our front range, but given that we have Colorado and Wyoming, a bulk of our Colorado and Wyoming area is actually classified as rural or even the word frontier, which might be a word that's unfamiliar to some folks maybe in the northeast.

A frontier county is used to designate a county that has fewer than six residents per square mile. The national average for population is actually closer to 80 residents per square mile. Given that we have such a large geographical range between Colorado and Wyoming, we did institute a statewide courier a few years back for all the birth hospitals for their first screens.

Next slide, Thalia. We are a two screen state and we recommend that the second newborn screen be done at 8 to 14 days of age. That second screen that we do in Colorado's actually an abridged screen. We really only, again look at the endocrine disorders as well as the Hemoglobinopathies and measure phenylalanine using the old chemical studies.

We have published a few times out of our state that 20 percent of our congenital hypothyroidism as well as our CH were found only on second

screen so I listed those papers that have been written by our endocrine and our public health folks here.

We do a majority of our follow-up actually utilizing our sub-specialist directly. We contract with these sub-specialists. Most are working at the Children's Hospital Colorado and that newborn screening lab actually calls out the abnormal screens to the sub-specialist and it's actually the sub-specialist that will speak directly to the PCPs, nurses and other folks as well as the families.

This really offers, being that I was over the metabolic clinic for the last ten years, there are a few benefits to this. It really allows for a provider to provider consultation. It also allows for good continuity of care.

As a genetics counselor over at the metabolic clinic, I would get the call about the abnormal newborn screen in the mornings from our state lab and I would be the one that would directly talk to the PCP as well as eventually talk to the family that day.

Then I was also the one who would often see the families when they first came to our metabolic clinic so again, it was a good continuity to be involved right in the beginning, to know about that abnormal newborn screen, but also make the diagnostic recommendations and even go as far as seeing the families when they came through our Children's Hospital.

We charge $92, which covers both screens. A large amount of that money actually even goes back to the sub-specialists that support our metabolic clinics and our sickle cell clinics and cystic fibrosis clinics and others. It also covers a bulk of those confirmatory studies so that when we are making recommendations that the studies are often covered for those families and we don't have to worry about things like insurance issues.

Next slide, Thalia. Just some stats about us. The number of births in Colorado for 2013 was 65,000 and I also included the number of births from Wyoming, but when we go ahead and we look at how many first newborn screens we received in Colorado, we actually see a little bit extra and we don't see as many Wyoming first screens that match that birth prevalence.

In saying that, I will say that there are a few hundred babies that we know that are Wyoming residents that are actually born outside of Wyoming. Because Wyoming is so rural, if it's a high-risk pregnancy,

they're almost always referred to a tertiary care center, which is often here in Denver or sometimes families even leave the state and go up to Montana for newborn screenings.

It makes tough for Wyoming newborn screenings to really track those babies down to make sure that they did, in fact, get their screen. The other is just reflex the Native American population that we will also screen for.

In looking at some of the questions that Thalia had asked us to answer, we don't have a formal process for tracking refusals. We do, over the years, have encouraged that hospitals themselves document this internally just in case there's ever an issue with a family's ... With litigation or such that they really should document that a family had been offered the screen, but had refused it.

In looking to pull together the numbers, they also don't track the infants that died prior to screenings. Nor do we do such a good job of seeing if there are Colorado residents that were born out of state that had their newborn screenings done elsewhere.

Next slide. This is our conditions that we identified in 2013. As you can see, I didn't have the ... CH has such a low number. I don't believe we picked up any in 2013. That was on the second screen, but for hypothyroidism, there was seven out of those 34 that were identified that were actually only found on second screens. You can see cystic fibrosis.

We did not have any diagnosis of SCID that year. We had started in 2012 and our first abnormal screen in 2012, about a week after the screening actually turned out to be a patient who actually had SCID, but since then we've had a relatively low hit rate for some additional diagnosis of actual SCID. Then our Hemoglobinopathies and then you can see our inborn errors of metabolism.

Next slide, please. Of course, there are other conditions and secondary findings that we find. The metabolic clinic, we'd often on our fog labs find a condition that we considered to be siglu, which is considered to be benign, but it's an elevation of C4.

Then there was always the issue with the inborn errors of metabolism of diagnosing maternal diseases. We found two moms with 3-MCC that year and then often we would have carriers and we know in Colorado that VLCAD cutoff is actually quite on the low side so it was not uncommon

for us to have so many kiddos with VLCAD that were diagnosed eventually as carriers after a good extensive workup.

We don't have any inborn errors of metabolism that were thought to be missed that year, but of course over the years we have missed some kiddos such as a kiddo with Glutaric acidemia. Another kiddo with VLCAD, another kiddo with LCHAD so there are some fatty oxidations that we've missed as well as organic acidemias over the years.

Cystic fibrosis has a number of carriers that we did using the IRT/IRT-DNA and then those are the Hemoglobinopathies that were diagnosed as either a trait of the hemoglobinopathy and a bulk of those were actually with the sickle trait.

Next slide, please. Some exciting and new developments for us in the coming years will be ... Recently in 2014, we actually moved our newborn screening follow-up staff from our main health department building over to the lab and at this point, our follow-up staff consisted of one person and she came over, Laura Taylor, to join the lab in the beginning of 2014 with the hope that maybe there'd be some improved collaboration and streamline of newborn screening follow-up.

Moving it over to the lab, though, also allowed us to expand our follow-up program with the addition of me as well as an additional FT that we'll be hiring in the coming weeks. With that expansion of follow-up, we've been able to really shift gears a little bit more and in the coming year, we really hope to focus more in education. Sorry for the spelling error. QA/QC, as well as data collection.

Coming up in 2015 as well as moving to 2016, we are going to consider expanding our newborn screening panel. In Colorado, for expanding a disorder that's picked up by a blood spot, we typically go through utilizing our advisory committee first and then moving that to the board of health. Tomorrow at our first advisory committee meeting of the year, we will be asking the committee to vote on three different disorders. Pompeii and PS1 and [inaudible 09:21] dystrophy, adrenal dystrophy.

If the committee says yes and we're going to do those each as a separate vote, for whichever of those disorders that the committee votes yes on, then we will be going to our board of health later this spring with the thought that if the board of health gives us the okay that we could begin testing in July 2016, the earliest, but even seeing that number may be even get closer to 2017.

For CCHD screening, because it's a point of care testing and not done here at the laboratory, we actually have to go through legislation to have that added so we know there's some active groups in town that will be bringing a bill to our Colorado legislation during the 2015 session.

We're not sure of the time frame as the last time we had used legislation was for the addition of hearing screenings so we'll have to see the time frame. Once that comes up, we'll get started again.

That's going to be tricky for us because given that we have a number of birth facilities at 5,280 or a mile above sea level, but even more importantly, there's a few percentage of our birth population that's above 7,500 feet so that'll be a little tricky for us, both for follow-up, but also instituting CCHD screening.

As far as for quality assurance projects, in 2014 we began a pilot project with our Colorado hospital association and hospitals to improve the timeliness and that project focused on education, IT and transport.

Moving that project forward, we were very lucky that Ustaff selected us one of the states, along with Wyoming, to participate in the new staff coin project, which gets kicked off this Thursday so we're very excited that we will be traveling to APH [inaudible 11:05] this week to, again, focus on timeliness for Colorado and Wyoming alongside of us.

I believe that's it. Thank you.

Thalia Wood: Yeah, thank you very much, Erica. That was a great overview. We're going to move our hats. In the absence of time, John, you want to go ahead and take over? Don't forget to star seven to unmute.

John Thompson: Can you hear me?

Thalia Wood: Yes, I can.

John Thompson: Excellent. Well, happy New Year, everyone. Just want to give a shout out to Carol Johnson, who's co-chair of this work group and then the whole work group for short-term follow-up for the great job that we were able to do this past year in 2014. I think it was a lot of fun working together and special thanks to Thalia and News Steps crew for their support and guidance along the way.

Next slide. Towards the end of last year ... Sorry, the last webinar in last year, Thalia ran in the important topics poll and two topics rose to the

top. The first was assuring every baby gets screened and the second one was reducing time to diagnosis and treatment. Our work group discussed both of these hot topics during our last planning call and all of the unveiling, which [inaudible 12:26] out of that discussion today.

The topic of most concern, assuring that every baby gets screened, has been receiving a lot of attention at the state and federal levels. We want to highlight successful strategies in future webinars.

In discussing the second-most vote-getter, reducing the time to diagnosis and treatment, we recognized that this topic spans the system from assuring that every baby gets screened all the way through testing, diagnosis and treatment so in each of the six webinars this year, we will be visiting this theme.

My former boss, Mike Glass, used to the director of Washington State newborn screening program, gave the keynote address at last year's symposium. He likened a baby with a newborn screening condition to a time bomb. If the system works quickly, we can defuse the bomb before it goes off. Delays can have devastating consequences.

Next slide, please. The time starts ticking when the baby's born. Our daughter was born last year. The newborn screening specimen was collected in the recommended window, sent to the state lab and all the results were available before she was four days old, except for the hemoglobin, which took one extra day. Thankfully her results were unremarkable. The system works smoothly, perhaps because they knew that I worked for newborn screening. We need to ensure the system works without a hitch for every baby.

Next slide, please. This slide is a general timeline that we use when we educate our health care providers about newborn screening. Birth attendance, whether they be at a hospital, birth center or at home, are critical pieces of the newborn screening system.

They need to collect a good quality specimen at an appropriate age and ensure that the blood is transported to the newborn screening lab in a timely fashion. The laboratory needs to operate efficiently and produce accurate results. Follow-up must be effective in reaching and convincing primary care providers to follow our recommendations.

Front line staff at clinics are challenged with establishing contact with the families, reporting results and ensuring that families bring their baby in for proper follow-up testing. Diagnostic testing must be accurate and

efficient and once the diagnosis is established, the baby needs to be treated without delay.

Next slide. There's a lot of places where this can go wrong. The old adage rings true. The newborn screening chain is only as strong as its weakest link. This year, we'll treat you to six webinars where we'll examine the newborn screening chain of events with a focus on providing information and resources to help your program strengthen its newborn screening process.

In considering short-term follow-up, some of our challenging cases are the babies that have abnormal newborn screening results who have some medical condition other than the one for which we made the referral.

In Washington State we call these true-positive, non-panel conditions. Sometimes it takes months to establish the final diagnosis. Often, the theory behind newborn screening is a simple idea, but the reality is complex.

Next slide. A good example of this is the test for T-cell receptor excision circles, known as TREC, to detect severe combined immunodeficiency or SCID. The simple idea is that zero TRECs means that the baby has SCID. The reality is that through screening, the number of babies identified with non-SCID T-cell lymphopenias outnumbers the babies with classic SCID by a factor of about eight.

SCID newborn screening is still relatively new and is the main focus of our webinar today. I will pass it along to my friend and colleague, Suzanne Skoda-Smith. She's an immunologist here in Washington State and she'll share some of the things we've learned in our first year of SCID screening related to this complex reality of identifying and treating babies with non-SCID T-cell lymphopenia.

Thalia Wood: Thank you, John. Suzanne, have you unmuted your phone?

Suzanne Skoda-Smith: I did so just now. Can you hear me?

Thalia Wood: yes, we can. Thank you, Suzanne. Go ahead and start.

Suzanne Skoda-Smith: Great. Thanks, John. I'm not signed in so Thalia, I'll have you do the slides and I'll tell you what you should be seeing. Some of them, I apologize are motion slides so you may have to click a few times.

The challenges with short-term follow-up for SCID really don't come for us with the patients with true severe combined immunodeficiency. They really have come with the non-SKID T-cell lymphopenias in our first year of screening here. We don't have the volume that the states like California have. We're about 80 to 90,000 births a year so that's pretty manageable.

Most of our screens come back really fast. Less than seven days and I venture to say our average is perhaps around four days. We really haven't had the problem with babies getting C and D from the time they're born to the time that we pick them up on screen, but we are a two screen state as well and one of our challenge has been that the first screen may be normal and the second one may be precipitously low.

It also highlights that some conditions such as a condition called charge syndrome can be really variable and it might be difficult to know the degree of T-cell lymphopenia those kids are going to have right off the bat.

Next slide, please.

Thalia Wood: Am I on the slide that says T-receptor excision circles. Is that the slide you want?

Suzanne Skoda-Smith: Stay there. I'm a slide ahead of you and I'm just going to say one word about the rationale for early detection of SCID. Since this is a relatively new screen, there's data from quite some time ago showing that there's a tremendous survival advantage if babies receive definitive therapy which is most often a stem cell transplant before three and a half months of age and that survival goes from in the 60 percent to in the high 90 percent.

Newer data that was just published in New England Journal through the PIDPC, which is a consortium of immunologists, looked at a retrospective study of 25 centers and they confirmed that survival and cure for SCID was high, regardless of donor type, if these babies were transplanted early. The key is to transplant them before they get infection.

You are now on T-cell receptor excision circles. Stay on that slide for right now. Part of the challenge in instituting this test is there wasn't a standardized approved testing method by the FDA. Jennifer Puck will be talking to you, certainly championed this and made this test a reality.

I remember her begging and borrowing people for Guthrie cards so that she could prove that a TREC or a T-cell receptor excision circle was a good surrogate marker for naïve T-cells and indeed it is, but this lack of FDA approval has impeded some states.

In fact, when we started screening, we used testing based on the Wisconsin method. They were the first state to a pilot screen and Tim Davison, our lab, really tweaked the performance characteristics of our testing.

We had to set what we thought would be a negative TREC value and we did that by going back and interrogating the cards of babies we had in the past with known SCID and our target came out at zero to 20 TRECs per microliter of blood. This is what the California paper would call an urgent positive. TRECs this slow would be referred immediately for confirmatory testing.

Go ahead and do the next slide and it should say tagging cells for flow cytometry.

Thalia Wood: Correct.

Suzanne Skoda-Smith: We actually created our own confirmatory panel. You can get lymphocytes subset analysis by flow cytometry, which counts your B cells and your T cells at a number of labs and even reference labs, but we wanted to capture our state's population of babies that were screened so we actually created a new orderable test in our lab called the SCID newborn screen confirmatory panel.

Had to be done on a small amount of blood so we only asked for a mil and this gives us the white count, the differential. Lymphocytes subset analysis, which counts the T cells, B cells and then K cells and then it gives us the marker of immature T cells, which is called CD4CD45RA.

We had really good success in getting these samples. We've even gotten an overnight sample shipped to us from a really rural town in Oregon and we've been able to actually do the confirmatory testing in our own lab.

The next one might give you a little trouble, Thalia. You might have to press it a few times. It says flow cytometry. We established our confirmatory cutoff based on the literature and on some of the screenings that had been done in larger states like California.

Our T cell cutoff or our C3 cutoff of the absolute number's 1,500. We actually looked at the literature and looked at a study that Bill Scheer did with the pediatric AIDS clinical trials group, establishing normal percentages of immature T cells in populations of normal [inaudible 23:12] and we chose 20 percent as our cutoff there.

Going to have you go to the next slide, which says Wisconsin date of first three years. Wisconsin piloted this test for a couple years before it was suggested to be added to the national panel. Wisconsin has a population pretty much like Washington, about 80 to 90,000 a year and this is their first few years of data.

Of the 72 infants who had abnormal TREC tests, over half of them had a normal confirmatory screen. Of the ones that had abnormal flow or an abnormal confirmatory screen, almost 60 percent of them were what we call a secondary T-cell lymphopenia.

This is a condition that is not a primary immunodeficiency, but can make your T cells lower and some examples are third spacing, anasarca lymph arctic anastasia and there's a very good list of conditions that can cause secondary T-cell lymphopenia, things that are known.

Of the 14 patients who had abnormal TREC and abnormal flow, they were followed and they were divided in about a third. A third of them had reversible T-cell lymphopenia and they had to be followed to determine that.

About a third of them had another primary immunodeficiency associated with T-cell lymphopenia, but not usually requiring stem cell transplant or other intervention like SCID and those were DiGeorge syndrome patients. Then again, about a third had true SCID or a severe T-cell lymphopenia that required therapy.

Knowing that, next slide, please. This should say Washington SCID newborn screen experience for six months. We were interested to see with a pretty broad cutoff, what we were going to get in our population. You'll see that the first four babies were in our cardiac intensive care unit and I think they thought I was stalking them for awhile.

The first baby was a hypoplastic left heart who did not get picked up before they went home. Went into cardiogenic shock at home and by the time they came in, we're in multi-organ failure so that baby had injuries incompatible with life and died.

The next three babies were another type of T-cell lymphopenia, DiGeorge syndrome babies and they've been followed and have moderate T-cell lymphopenia and aren't likely going to need any additional therapy other than monitoring.

The next baby, a probable charge syndrome baby, you'll see did have a TREC of zero, but that baby also had so many congenital anomalies that they were not compatible with life and that baby died.

Our first true-positive was an unknown SCID genotype who had an abnormal TREC on day four of life. We had the screen back the next day confirming it. He was transplanted at two months of age, out of the hospital at three months of age and is back to his community at seven months of age. I think our first positive went about as well as we had hoped it might go.

The pulmonary embol adjectatious in micro preemies with sepsis are all conditions that have been associated with decreased TRECs and you'll notice there that we have the phenomenon of dropping TRECs, which in a two screen state, we sometimes see.

The last male listed there with charge syndrome has had to be followed for awhile. He had a twin who died in utero and when that twin was autopsied, they were found to have no thymus. This baby's TRECs were pretty darn low, 12 and 22. Getting pretty close to what we define as zero so this baby had an ultrasound to make sure he had a thymus.

Had low T-cell numbers, but good proliferation and I just saw him at about seven months of age. He still has quite a low T cell number of about 500, but he's not required antibody replacement and his proliferation is normal. He's taken several months of follow-up to see which way he's going to go in immunologically. It doesn't look like he's going to need a bone marrow transplant.

Next slide, please. There are perils and pitfalls in the non-SCID T lymphopenias. So far in our series, DiGeorge syndrome and charge seems to be the most frequently found T-cell lymphopenia that we would consider a primary immunodeficiency.

There are lots of secondary causes that can give you T-cell lymphopenia. In our series, we saw plenty of third spacing, multi-system illness, pre-maturity and multiple congenital anomalies.

You do have to be a bit of a detective and take a good history, which is where the provider communication is important and some babies with T-cell lymphopenia and no known reason for it are going to require some follow-up to see if they resolve.

I'm going to give you two quick cases and then I'll end, just to give you an example. Case one is of a sick preemie. This was a 600 gram, 25 weeker and for some reason, this baby got four screens.

I think it was probably because it was early and they got transferred from another hospital, but you'll see that the TRECs are really quite good on the first three screens and then around a little bit short of two months, they dropped precipitously. There was no family history of SCID, but this was a sick preemie, with pretty much everything that can go wrong with a preemie. There was no congenital cardiac disease or unusual infections, though.

The problem with getting the screen on this baby is that ... The confirmatory screen is that the absolute lymphocyte counts were chronically low so we knew if we got close cytometry, we'd get an abnormal.

There were also problems of sending labs due to volume restrictions so we followed this baby closely for a month, kept tabs on the clinical picture and when the absolute lymphocyte count popped up to normal at about three months of age, we did send confirmatory flow and it was normal.

The additional info was that this baby was found to have acquired CMD. Of course, if this baby had had SCID and gotten CMD, they wouldn't gotten rid of it and this baby did not get generalized disease and did not get treatment and resolve their CMD.

That's one of the quandaries you can get into. We paid attention to this dropping TREC because this immunologist, one type of SCID may not show up on the first screen. I don't really know what the data for that is. It's going to be interesting to see if we do find ADA deficient SCIDs on second screens and I'll be interested to hear if Jennifer has any data on that.

Then finally, the child that goes to cardiac surgery. Yes, they're fine. It's removed. It's going to have a dropping TREC so you just have to get a good history and know that's the reason for it. Some of these babies will also be on ECMO from the get-go and we've actually found that people

have drawn the screen from an ECMO line and of course they come out with zero TRECs.

We also had an instance where a zero TREC was called in and I was getting ready to call the physician when John called me back and said that the screen had a dull hemoglobin pattern on it. The people who'd done the blood spot had used a vial of the mother's blood to do the blood spot.

I think that the challenge for us is going to be how do we follow these children who are going to end up having reversible T-cell lymphopenia most likely so that we don't over test or under test them.

Alright, back to you, Thalia.

Thalia Wood: Thank you very much. That was very interesting. We'll hold questions till the end. We'll move right into Dr Puck's presentation so Jennifer, can you do a star seven to unmute your phone?

Jennifer Puck: I think I did. Can you hear me?

Thalia Wood: Yes, you did. We can hear you, thank you.

Jennifer Puck: Okay, and can you advance the slides?

Thalia Wood: Yes.

Jennifer Puck: As Dr Skoda-Smith said, I'm working with the California newborn screening program. I'm also an immunologist at UCSF so much of what I'm going to say is similar to what she already said, but I think it's good to see a couple of different angles coming out with quite similar feelings about what's going on with SCID newborn screening.

This first slide just shows the definition you would get if you opened any textbook, including one that I wrote, about SCID in the past. We always recognize these infants have absent T cells that they've made themselves. Possibly maternal T cells might get into their circulation.

They also have inability to make any specific antibody production and so the reason it's called combined immunodeficiency is that it hits both the T cell or cellular and the B cell antibody or humeral immune system and without either one of those arms of the immune system working, recurrent infections set in very soon and babies start to experience

weight loss and they just gather up infection as those next two points show.

They basically can't get over anything. If they happen to get a rotavirus live vaccine, they get rotavirus diarrhea and they don't become virus negative for months until a T cell reconstitution with a transplant is actually successful.

It's not hard to imagine that these babies die early with this accumulation of increasingly severe infections unless we can establish a working immune system and this is most often done by giving them a bone marrow transplant from a healthy, matched person, but in the case of [inaudible 34:15] deficiency, the enzyme is available for injection and can be a replacement.

Gene therapy is experimentally available for ADA, SCID and now x-linked SCID and you've already seen this improved outcome data from the last presentation so if we could go to the next slide.

There are many different genes that could be defective and lead to SCID and so I listed them here just with some of the different categories. Some are sight of [inaudible 35:00] receptors and some are involved ...

That would be the first two stripes here and then the ones in the middle in blue have to do with the recombination of T cell receptors and the B cell antibody genes. T and B cells are the only cells in the body where in addition to RNA splicing, there is DNA splicing.

The receptors for all these different particles in the environment that have to be neutralized by the immune system, the receptors are arranged as alternate little sections and one section is chosen for the mature gene and the mature receptor.

If this process of selecting and cutting and pasting the DNA to make these mature receptors, if that is interrupted, then cells don't continue to mature and SCID is the result. By the way, those TRECs are bi-products of this T cell and B cell rearrangement of these DNA receptors.

Then we already mentioned the enzyme at the [inaudible 36:27] and then there's some very rare and some of them syndromic disorders associated with SCID at the bottom. We're not done yet. There are many infants who are coming to light with SCID, some of them through newborn screening, where we don't yet know a gene.

Next slide. You've already seen a picture of this, too, this recombination process that makes the mature T cell receptor gives a circular bi-product, which is the TREC. At the bottom here, you can see that section with the TCR delta locus in the brown boxes.

That is lopped out in developing T cells in the thymus that are destined to express the alpha receptor and the delta receptor is just sitting there in an intron of the alpha receptor so it gets removed by one of these recombination events and the leftover DNA turns into a circle and because this happens very frequently in cells that are going to become alpha beta T cells, about 70 percent of those cells actually have this circle.

That's a quirk of human immunology that makes the TREC a very nice analyte. It's present in most of the cells and therefore you can do a PCR reaction and detect it so that the signal to noise is quite substantial.

Next slide. These algorithms, every state develops and I'm sure you have them in your own states. This is the way California works after an initial TREC [inaudible 38:32]. If it has a number of TRECs that's over 40, no further testing is done and if the number is below that, then it's repeated with a control DNA, an actin test.

California is a one blood spot state for ... I guess they don't have a routine second spot, but they ask for a second spot if there's a incomplete test or these blue boxes called DAF is DNA Amplification failure in our lab.

Then built right into the California screening program and I was really happy to hear that the Washington program has developed a similar thing. There's a CBC and lymphocyte subset panel that is run.

In California, it's contracted out to a single lab so that all of the testing is sent to the same place and this lab is a quest lab, which is the same lab that does the follow-up for the metabolic screening so that draw stations are all over the state and already established.

That makes it easy to collect these samples. Having them all run at one lab is very useful and important. Then the other thing is having these mail-ins organized so that it's not a big hassle. That's also very important.

A third thing is that we've asked this contract lab, when they're running the T cell, B cell counting test, they also put a drop of that same blood sample onto a filter and send it back to the state lab for a TREC test and that allows measurement of the TRECs on a sample where you actually know the number of T cells that are present.

That's important because we don't know how many T cells are there in that initial dry blood spot because the baby didn't have a lymphocyte determination at that time. This has allowed California to match up TREC numbers with T cell numbers on an ongoing basis to see whether they're cutoffs are appropriate and so on.

Next slide. As you just heard about for Washington and I think this is really one of the main things that I was asked to address today, is we have to figure out when the TRECs are not normal and when the T cells are low, what is going on with this baby. We have developed different classification categories for these babies and they've been adopted by the R4S system and we actually work these out in collaboration with the PIDTC, this primary immunodeficiency treatment consortium that is funded by the office of rare diseases and NIAID.

The categories that have been defined are listed here and the first one is typical SCID. This is defined now as less than 300 and autologist means you made them yourself so these are less than 300 autologist T cells per microliter and a poor function, which is measured as proliferation to a mitogen PHA.

Now if there are maternal cells present, those maternal cells add to the total number of T cells and there can be a lot of them, but they don't count so you have to look for maternal engraftment and actually that is useful to look for because maternal cells arrive in the baby's circulation in every single baby who's born, but baby's with normal immune systems get rid of these maternal cells very promptly.

Babies with SCID cannot get rid of them so the presence of maternal engraftment is an indicator of typical SCID. Of course a known gene mutation if you can find that out is also an indicator and one of the big problems currently is that it just takes too long to get the genotyping done for these rare conditions and as I showed, there's so many genes.

It would be nice to have a quick gene panel test and we don't really have that yet. The turnaround time ranges from six to ten weeks, which is just way too long to wait for a SCID baby.

There are instances of leaky SCID, the next category down in which the same genes are involved, but the mutations are hypomorphic so that allows the baby to make a few T cells and sometimes a few is worse than none at all because these T cells can attack the baby's own tissues and cause omen syndrome.

That's the next line down. These babies get rashes and big liver and spleen and they can get GI malfunction and all sorts of other complications from these rogue T cells that are not regulated properly.

Those first three categories. The typical, the leaky and the omen syndrome, they generally require establishment of a better immune system by means of a transplant or gene therapy or enzyme therapy so those are the medical emergencies that have to be treated right away.

Then the four categories in the lower part of this slide are things that in their severe form definitely do need immune management and need an immunologist watching over the patient, but on the other hand they can self-resolve.

One of the issues is can immunologists be lined up all over the state to take care of these babies when they are diagnosed by newborn screening and actually in California we have such far flung babies where immunologists are not there and by the way, the board certification in immunology is combined with allergy and it's adult and pediatric.

You have to know that by far the majority of board certified people in the specialty of immunology are actually going to be adult allergists. You can't just trust any old person who's not familiar with these very rare inherited genetic disorders to see these kids.

In California we started taking care of that by encouraging the babies that we need an immunologists to follow to come to one of four centers of excellence that are established in the state and then an immunologist at one of those four centers can work with the local pediatrician or a local immunologist/allergist for further management once we've got a handle on the severity and what is going on.

These conditions I think that were already mentioned in the last presentation. The first is syndromes with low T cells and these would be recognized clinical syndrome in which T cells is part of the spectrum such as the DiGeorge 22Q deletion.

The next category is secondary low T cells and these are congenital malformation diseases or processes that don't have an immune component of their own, but they can cause T cells to be low.

When I think of these, I think of congenital heart disease as the most common one, but also something like congenital leukemia where the bone marrow is just completely busy making leukemia cells and not

making cells that turn into T and B cells. We also recognize some infants with extreme preterm birth don't have normal TRECs or T cells very early on and then they normalize over time.

Then the last category, of course as newborn screeners know and love, there's the idiopathic T cell lymphopenia that shows up and we define this carefully in California and I think you watch out because different states have different cutoffs for what they consider low T cells.

This would be persistently low T cells that is for more than three months. In California less than 1,500 T cells per microliter. In addition, they should have some sort of functional T and/or B cell problem and also not have a defect in a typical SCID gene as listed above.

Then sometimes a cause of this low T cell count does surface and in that case, the infant is moved out of this category into the category where they really belong.

Next slide.

Thalia Wood: Excuse me a minute, Dr Puck. We only have about nine minutes left and we need to leave a little bit of time for questions if you want to try to move a bit faster.

Jennifer Puck: Let's jump the next slide. What's listed in black there was the same definitions that I started with and then the red shows that these are ... We have to re-do the definition because the babies don't get infections and that's the whole point of newborn screening so these things that used to be defining are no longer there to help us out and we have to rely on the lab values.

Next slide. This is just summarizing what has turned up in California after four years of screening nearly two million infants. As was mentioned before, when infants in California had low TRECs and had to be sent for flow, somewhat more than half of them had normal T cells, so 57 percent.

The other categories that are shown in this pie chart actually have T cells under 1,500 so typical SCID and leaky SCID and omen syndrome are the red and orange ones and the idiopathic ones that we have to follow are next. Then the syndromes, the secondary causes and preterm.

You can see with the numbers on the left-hand side that this is actually our screeners favorite condition because in California only 1.3 infants per

10,000 requires the follow-up flow cytometry test. The positive predictive values really excellent for TREC and of the T cells of 43 percent so again, a very high predictive value actually have low T cells that we think are low enough to pay attention to.

Another comment on the 1,500 T cell cutoff, which is the one California and Washington and several other states use, but not all. We arrived at this by saying at what level should we intervene and tell that baby not to get a live rotavirus vaccine and we felt that babies with more than that number of T cells were probably safe to get the rotavirus vaccine and so that was one of the reasons for establishing this.

We don't want to go bother babies where we're not going to actually do any intervention and just sit back and watch them. We didn't think that was fair.

Next slide. This is just a quick comparison of historic data on the left to the kind of data that we're actually finding once we institute newborn screening on the right. You can see that the old data, which is from transplant centers, had really different ratios of the different genotypes for SCID with half of the cases being x-linked form.

If you look at what we're finding by screening, we're finding only more like a quarter of the cases being the x-linked form. Many more of the cases due to this RAG1, which you can see right below that and that's because several of them are leaky SCID that we wouldn't have found perhaps as early in life before because they may last a little longer before getting severe infection. Another interesting thing about this pie chart on the right is how many unknown genotype SCIDs there still are.

Next slide. I think as was stated before, the outcome have turned out to be excellent.

Next slide. Can we get the next one? This is just a list of some of the non-SCID conditions, including the multi-system syndromes. The DiGeorge 22Q deletion is the most common. Interestingly, Down syndrome or trisomy 21 is next. Ataxia telangiectasia and charge syndrome are also in that category.

Of the secondary ones, you already heard Dr Skoda-Smith mention these, the cardiac anomalies, other congenital anomalies, vascular leakage, neonatal leukemia and the extreme pre-term birth being another category.

Next.

Thalia Wood: Actually, I'm going to skip to your conclusion slide because we do have one last presenter who's just going to talk for a minute so we just need to wrap it up here.

Jennifer Puck: Okay, and I did want to say because Suzanne Skoda-Smith asked about the ADA deficiency, we've had one partial or late ADA and that was not detected early and wouldn't have been detected later either by TRECs or T cells. Those are T cells that fall later in life. I'm done.

Thalia Wood: Great. Thank you so much. Just to let everybody know, these slides, of course will be available, the complete set of slides on the web later. Real quickly here at the end, we wanted to talk just for a minute at the end about what happened in New York and Beth, you want to go ahead and take that over real quickly.

Beth Vogel: Yes, this will be very brief. Thank you. Can you hear me, Thalia?

Thalia Wood: Yes, we can, thanks.

Beth Vogel: Thank you. This is Beth Vogel. I'm the follow-up supervisor at the New York newborn screening program. We had quite the weather event in western New York in November. Parts of that area received upwards of seven feet of lake effect snow. You can see just some images on your screen of what that looked like.

The newborn screening program's response to that storm. We worked with our carrier to identify the impact at hospitals so they told us which areas they were unable to get to. That was a total of seven hospitals. We contacted all of those hospitals to determine the number of newborns in the nursery and the NICU and also determine how many specimens they had currently that had been collected and were waiting for pick-up.

We then monitored the number of those specimens we received each day. There was only one day where we could not get specimens from any of the hospitals. Most every other day of the storm, either the carrier was able to pick up or we were able to find an alternate way to receive the specimens so overall, I think that we able to handle it pretty well and people in western New York at the hospitals and the carrier were very helpful as well.

Thalia Wood: Great, thank you. That was an interesting [inaudible 57:02] that you had to deal with there. We wanted to just hear about that for a second.

We do have a couple minutes left. If people have questions, just star seven, unmute your phone and you can ask questions to the speaker or you can type them into the chat box.

Not hearing anybody come on, I'm going to go ahead and do a couple real quick polls here at the end. The work group had questions for you. You can answer more than one of these.

Which ways would you be interested in collaborating for follow-up? You can choose more than one of those responses at this time. You don't have to just choose one if you want to choose one now or more than one. We do use your responses to help us with our work group. I have one more question after this real quickly. I'm going to go ahead and show the survey right now with the results.

Great. Thank you so much for participating in this poll. I'm going to go ahead and stop this poll, go onto the last question. Like I said, we will use this information. This is what tools would you like to see developed? Again, you can answer more than one on this one as well. Okay, thank you.

John, any final words before we get off today? John, are you still on?

John Thompson: Are you there?

Thalia Wood: Yes, we're here.

John Thompson: I was muted, sorry. I just want to thank all the speakers for their great presentations. Appreciate all your help and want to reiterate that if people have ideas of things that they want to do or think about or help with, please just let us know.

Thalia Wood: Yes. Thank you, everyone. Thank you for your time. Thank you to the speakers. You were great and we'll be in touch in a couple months. Thank you.