Alpha Thalassemia Webinar Part Two Transcript

Careema Yusuf: Perfect. Welcome everybody. Before we begin, I’d like to just announce that this webinar has been supported through a cooperative agreement with CDC and that none of the views that are presented here are representative of the Department of Health and Human Services or of CDC. I’d like to hand it over to Tim to begin the webinar. Thank you.

Tim Davis: Thanks, Careema. Hello and welcome again from the state of Washington. On behalf of the APHL Hemoglobinopathy Workgroup, I would like to welcome you to part two of our webinar series on alpha thalassemia. My name is Tim Davis, chair of the hemoglobinopathy workgroup and lead microbiologist in charge of hemoglobinopathies skid and molecular testing at the state of Washington.

During our first webinar, you heard Dr. Aguinaga and Dr. Bender discuss the clinical aspects of alpha thalassemia. Dr. Aguinaga spoke on the clinical forms and defined both deletional and non deletional types, inheritance, treatment, and clinical detection. Dr. Bender addressed the need for detecting alpha thalassemia in newborn screening and how early detection can prevent potentially devastating outcomes in the case of hemoglobin H constant spring. He also addressed how when hemoglobin H is not detected in the newborn, it can lead to unnecessary treatments and diagnostic complications. Also, how the reporting of alpha thalassemia trait can be useful in preventing unnecessary iron supplementation and avoid complications, which create an odyssey of misdiagnosis. Finally, both presenters stressed the greater ease and cost savings associated with diagnosing hemoglobin H in the newborn period.

Today’s webinar focuses on alpha thalassemia newborn screening in the US. There are three segments of today’s presentation. Alpha thalassemia detecting and reporting, screening algorithms, and a summary of our alpha thalassemia reporting survey. Once again we’ve reserved time at the end of this presentation for questions.

Next slide. Okay. My talk is on alpha thalassemia detecting and reporting and newborn screening. During this presentation, I’ll discuss chain expression and imbalance, hemoglobin Bart’s percentages and cutoffs and hemoglobin constant spring. I’ve also added some information regarding follow-up for trait and hemoglobin H disease.

Next slide. In a normal newborn there are a total of four alpha chains that are expressed from chromosome 16, as shown in the gene cluster on the left side. Two chains from alpha one and two chains from alpha two. The alpha two chains are expressed at a rate of 2.6 times that of alpha one. This is due to the alpha two gene’s proximity to the major regulatory unit upstream from the alpha two sites. It’s closer so it produces more than alpha one.
On the right side of the slide is the beta-like gene cluster from chromosome 11. Two chain are produced and a combination of two types of gamma chains are produced, two beta chains that is. Those differ by a glycine and an alanine, although for this talk, they will be considered functionally the same. Therefore, the net output of both gene clusters is four alpha chains, two beta chains, and four gamma chains. The graph in the middle shows chain output as a function of gestational age. If you look at the gray rectangle in the middle, you will see that alpha and gamma production is high and beta is low. To the right of the gray box is where the switch occurs at approximately 21 days of age.

Next slide. Newborn screening specimens collected within the first few weeks of life will show high levels of fetal hemoglobin with a strong band or peak and low levels of adult hemoglobin with a weak band or peak based on chain expression from the previous slide. In this situation there is no chain imbalance and no alpha thalassemia. The ratio of alphas to beta like chains is one to one.

Next slide. This is an example of alpha thalassemia trait. On the left side, the red x's depict alpha genes that are no longer producing functional alpha chains either by deletions or non deleitional mutations. In this case the affected genes are in sis to one another since they lie in the same chromosome. An imbalance has occurred in functional alpha chains, which creates an excess of gamma chains and hemoglobin Bart's tetramers are formed. The reduction in functional alpha chains is alpha thalassemia. The three bands circled on the IF gel and the single peak on the HPLC are both hemoglobin Bart's.

Next slide. Hemoglobin Bart's is a tetramer of gamma globin chains. Once again you can see the red circle and red arrow point to the Bart's peaks and bands. Bart's is formed when there's reduction in functional alpha globin chains. On isoelectric focusing gels, Bart's presents as two to three bands. Actually there are always three, but the lesser band on the right becomes very faint depending on the number of alpha genes affected. This is also dependent on gamma chain expression as a child ages. On cation exchange HPLC, Bart's presents as one to three peaks. The first peak or set of peaks is often called fast, since it's the first to allude. The display of one peak or multiple peaks is dependent on the resolution of your HPLC system, which we will talk about later.

High levels of Bart's are more common in Asian populations. Big disclaimer here: this is a genetic disorder so it can be found in any race although Asian is the most common. Bart's causes severe hypoxia and Bart's hydrops fetalis syndrome. Hemoglobin Bart's has a high affinity for oxygen and prevents it from being distributed to the tissues, which is the normal function of hemoglobin. Also, it was discovered at St. Bartholomew's Hospital in London, which is where the name comes from. That was in the late 50's.

Next slide. What are the Bart's bands on IEF? Finding an answer for this was no easy task. There's no clear text reference out there. The go-to reference in the literature is a paper published in 1987 on the minor components of Bart's. They list them to be Bart's, acetylated Bart's, and glycated Bart's. Post-translational
modifications form acetylated and glycated Bart’s. I also reached out to [inaudible 00:07:16] and these bans were confirmed by [inaudible 00:07:20]. A while back they did an extensive mass spec study that confirmed the identity of these bands.

Next slide. Here’s an example of three affected genes for hemoglobin H disease. On the left side only one alpha chain is being produced, although it is being up-regulated due to the deletion of alpha two. As you can see by the circled Bart’s region on the gel, the bands are pretty intense and the HPLC percentage is high at 34.3%. This is a typical presentation of hemoglobin H in the newborn, in contrast with the previous example of alpha thal trait where the bands were not as strong. Also, as the child ages and beta chain expression increases, the Bart’s percentage will decrease. Beta chain tetramers will be formed, which is hemoglobin H.

Next slide. Before I take the plunge into percentages and cutoffs, it’s important to point out that most of the published percentages are from EDTA whole blood or cord blood and not dried blood. Also, any percentages from kids older than three weeks of age are going to be different than what we see in newborn screening. Finally, not all HPLC systems are created equal. Software integration, gradient profile, elution time, and resolution all play a factor in the number. More on this in the next few slides.

The percentages shown in the table are what we use or used to use in Washington and I’ll explain. The first scenario is the comparison of FA normal, the silent carrier highlighted by red rectangles and red arrows. The reason why I do not recommend reporting silent carriers is because there’s an endogenous level of hemoglobin Bart’s in newborns, which makes the line between one affected gene and none too difficult to reliably detect. It’s also clinically benign so for us it was a program decision to discontinue reporting them.

Next slide. This next scenario is the comparison of silent carrier with alpha thal trait. The separation between these two is better than the previous, but still not perfect. We placed our cutoff for alpha thal trail at 9.2% based on experience and a genotype phenotype correlation, which I’ll show in another slide.

Next slide. In this scenario we compare alpha thal trait with hemoglobin H disease. The separation between these is excellent. There’s a clearer separation between both of these than in the previous examples.

Next slide. I’ve inserted this slide to highlight the differences between two HPLC methods. Along with the BioRad, we used to use an in house extended gradient method. The BioRad has a 2.5 minute gradient as opposed to the run on the right with a 50 minute gradient. The extended gradient has excellent resolution and the hemoglobin Bart’s percentages are very different. The Bart’s percentage decreases due to the increased number of peaks because the percentage is calculated using the total area of all peaks. The point here is that whether you
use Primus, BioRad, or an in house method, your percentage results will not be the same.

Next slide. Here again we see the differences in percentages between the BioRad classic, BioRad VNBS, and extended gradient systems.

Next slide. This graph represents one year of BioRad Bart's percentage data from May of last year to April of this year here in Washington. Our primary method is IEF and all abnormal traits disease, including low to high Bart's are repeated on BioRad. Since we're a two screen state, we're looking at both first and second screens. The red lines represent Bart's percentage cutoffs of 9.2 for trait and 25 for hemoglobin H. We had eight hemoglobin H's reported during this time and they are represented in the circles in yellow. Notice how each group has the first dot higher than the second. Three screens were also collected on one of them. What you're seeing here is the reduction of gamma chains over time as the child ages. On average this is only a separation of a week, but the drop still stands out.

Next slide. This graph represents the same data in a histogram. Once again, I've shown the same cutoffs. What's interesting here is how the traits drop off at around 22.5% and there's a desert until you hit 25.7%. This gap is significant and in general this is what we see. The remaining hemoglobin H's are the large peak on the right. This gives us confidence in reporting hemoglobin H disease.

Next slide. This is a very small genotype phenotype correlation we did about ten years ago. We list the BioRad percentages with the corresponding deletional genotype. If I could do this over, I would have included normal Bart's percentages and in general a lot more specimens, however this study is still helpful. The cutoffs of 9.2 and 25 are in red. The blue dots are the Bart's percentages from the table on the left. I called the first category mixed, which basically means that there's a wide spread of percentages with no deletions found. This can be explained by the fact that point mutations were not tested. There also can be mutations in the regulatory regions that can cause alpha thalassemia. The second category is silent carrier with a median percentage of six. Category three is deletional alpha thal trait in trans with a median of 8.85. Category four is deletional alpha thal trait in sis with a median of 13.35. All lonely by itself is category five with a confirmed three gene deletion of 36%. What stands out here is that alpha thal trait in sis has a higher percentage of Bart's compared with trans.

Next slide. During our first alpha thalassemia talk, Dr. Bender mentioned the HS40 super enhancer, which lies upstream of the alpha two gene. The gene cluster on the left shows normal expression in purple of alpha one and alpha two. Alpha two has an expression again of 2.6 times that of alpha one due to its proximity to the regulatory region. The middle example is alpha thal trait in trans. On the left chromosome, alpha two is up-regulated because alpha one has been deleted. The same thing happens on the right chromosome so alpha thal trait in trans has a higher expression and lower percentage of Bart's
produced. The far right example of alpha thal trait in sis shows a net reduction of alpha chains when comparing it with the trans example because up-regulation does not occur. This is why alpha thal trait in sis has higher levels of Bart's.

Next slide. Hemoglobin constant spring. Hemoglobin constant spring is a point mutation on the alpha two gene and stop code on 142. There's a thymine cytosine switch that causes the stop code on to change to a glutamine. This leads to an extension of the alpha chain to 172 amino acids. This long peptide chain is unstable and there is very low percentage of protein present. It's detectable in low levels on IEF and not on HPLC using dried blood. However, if your matrix is EDTA whole blood, it can be picked up on HPLC systems. It's possible that capillary electrophoresis will also pick it up using dried blood. We see more of these on the first screen than the second. In Washington, we receive a first screen that is collected at approximately 24 hours of age. The second is collected at about seven days old. It's not uncommon to detect constant spring on the first screen and not on the second. We see this often and I'm not sure why. Hemoglobin H with constant spring is clinically more severe than deletional Hemoglobin H. This also applies to the other non deletional alpha thalassemia's, that for the most part reside on the alpha two gene. Also, the lack of up-regulation causes alpha one to produce less chains than deletional hemoglobin H, which is why it's more severe. Finally, hemoglobin constant spring is the most common of all the non deletional mutations.

Next slide. What does hemoglobin constant spring look like? Here's an example of hemoglobin H with E trait and constant spring. The two green arrows to the right of the E band are the constant spring bands. Faint but detectable. These bands are cathodal of hemoglobin C so they lie in an area of the profile that's pretty clean. In other words, there's less artifact bands in this region, which benefits detection. I've got a lot of confidence that when I see these bands, it's hemoglobin constant spring and we have the DNA results to back that up. You can see from the chain tetramers above the [camadogram 00:17:30] that two bands are formed due to the mix of beta and gamma chains.

Next slide. Here's an example of alpha thalassemia silent carrier constant spring. The Bart's percentage is pretty low at 7.6%. I train my analysts that when they see hemoglobin Bart's, they need to look cathodal of C for the constant spring bands. Something else noteworthy here is that there's no hemoglobin constant spring point on the HPLC chromatogram. If you look at the chromatogram, there's a slight blip where I've placed an asterisk. That's not constant spring. It's actually an artifact from the [kit lod 00:18:09].

Next slide. Here's another example of constant spring on IEF. This is an example of alpha thal trait at 17.1%.

Next slide. To confirm the constant spring mutation, we use PCR allelic discrimination. We have a smart cycler two system and even though it's ancient, it gives us what we need. We've also run them on the VS7 instruments where
we do our skin assay, but found it was better to keep the assays separate. There's a TaqMan MGB VIC probe for the wild type and a TaqMan MGB FAN probe for the mutant type on all our genotyping assays. The amplification plot is on the upper right. Both mutant and wild types amplify so this is a heterozygote plot. A strategy to test for hemoglobin H constant spring would be to confirm hemoglobin H by either IEF, HPLC or a combination of both. Then simply test for the constant spring mutation. Occasionally we pick them up this way even when we don't see the constant spring bands on IEF. This is very low volume confirmatory testing since hemoglobin H is uncommon. I've also placed an asterisk on the hemoglobin H just to indicate that we don't know if this is deletional hemoglobin H or a more severe non deletional form other than constant spring. This would be a good justification for sequencing the alpha two gene.

Next slide. I wanted to include some real world data so I asked Tennessee and Texas for their hemoglobin H annual positives over a ten year period. This allowed me to establish a rate so that all three program's data was comparable despite the difference in population. Washington detects 6.3 hemoglobin H's per 1,000 births. Tennessee detects 2.7 and Texas detects 4.8 interesting to note that out of the 6.3 hemoglobin H's detected in Washington, 10% of those had the constant spring mutation. I also wanted to find a way to make this data meaningful to all programs so I looked at the census data from 2010, which is in the middle of the ten year time period. The Asian population is fairly consistent with the hemoglobin H rate per 1,000 births. There is some predictability here.

Next slide. In an effort to leave no stone unturned, I've added a couple of slides on alpha thalassemia follow-up. For alpha thal trait, first a physician receives standard newborn screening results in the mail. They also receive a hemoglobin trait packet with the trait report above, a Bart's fact sheet, an alpha thal brochure, and local hematology and genetic counseling resources. The physician then informs the parents of the finding and shares the resource information with them. The text on the upper right describes the methodology and casts a wide net regarding interpretation of our results. It refers to the possibility that this child could either have silent carrier or a more severe form of hemoglobin H disease.

Next slide. For hemoglobin H disease, after the second screen, the physician receives a phone call from us, a faxed hemoglobin H packet including the memo above, a Bart's fact sheet, a hemoglobin H brochure, and local hematology and genetic counseling resources. They also receive the packet in the mail with the standards of care guidelines for thalassemia published by Children's Hospital and Research Center in Oakland, California. The physician informs the parents of the finding and shares the brochure and resource sheet with them. Our follow-up staff ensures that an appointment is made for a diagnostic evaluation with a pediatric hematologist.

Next slide. To wrap things up, we have a unique window of opportunity to detect alpha thalassemia during the newborn period. Once the child reaches the
age when the switch occurs between gamma and beta chain production, Bart's percentages drop off and it becomes difficult to detect alpha thalassemia. HPLC percentages must be determined from matrix appropriate samples and tested during the same time period, the same method. Excuse me. Studies that show Bart's percentages of cord blood, whole blood, kits greater than three weeks of age, and different HPLC systems are going to have different percentages than what we see in newborn screening. Silent carrier reporting can be problematic due to the endogenous level of hemoglobin Bart's in newborns causing an overlap between one affected gene and no affected genes.

Genotype phenotype. More comprehensive work needs to be done to improve specificity for alpha thal trait detection. It would be helpful for those that report alpha thal trait to be able to reference a robust study that gives a clearer picture of where the cutoff should be placed. There's a percentage gap between trait and hemoglobin H disease. Hemoglobin constant spring can be detected on IEF in the newborn. Mutation testing can also be done to confirm. Finally, RUSP inclusion as a primary method, where do we stand? Hemoglobin H was nominated back in 2010 and the committee found that there was no compelling clinical need presented and specified that there were evidence gaps in the literature. It remains a secondary condition listed as various other hemoglobinopathies. I'll have more on this at the symposium in September so stay tuned.

This concludes my portion of the webinar. Our next speaker is Joseph Ubalke. Joseph currently serves as the supervising microbiologist for newborn screening within the Katherine A. Kelly State Public Health Laboratory at the Connecticut State Department of Health. Welcome, Joseph.

Joseph Ubalke: Thank you, Tim. My section for this presentation will be for the algorithms of our testing in the lab. This presentation was prepared between myself and Dr. Chan of Florida Health Department.

The first thing that we want to look at is the screening methodologies that we have, which first of all, we can use isoelectric focusing in electrophoresis kind and the high pressure liquid chromatography. These are the two different ways we use. Of course, if you want to do more confirmatory, you can use the DNA. But in many cases, many states use the isoelectric focusing and some of us use the high pressure liquid chromatography. I want to indicate that you can use a combination of either the isoelectric focusing and the HPLC or HPLC and IEF, depending on which one the state wants to utilize first, and the other one as a secondary test. But for those who do also the confirmatory, they add the DNA to define and separate [inaudible 00:26:06] false positive results that we have.

Next slide. The first way of talking about hemoglobin, we had some charts that we have from this CMG. This one is about sickle cell, but at the present, we are dealing with the thalassemias, alpha and [inaudible 00:26:28] thalassemias. We'll focus on the thalassemia chart.
Next. In this chart from this CMG algorithm, you can see the different levels of results that we have. But what I want to focus on this is the percentage of the Bart's. In this case we have 25%. If it less than 25%, then no follow-up testing because it's insignificant to that state, but if it is greater than 25%, then further action is needed. One of the things is that every state, when you get the HPLC, which many states use the BioRad HPLC, but others use the other kinds of ... Like the Primal HPLC. But in the BioRad HPLC, it's already set in it, 26% or so for the batch level. But in our own state, we wanted to be a little conservative so we set it at 20%. Anything that is greater than 20% will flag and when it flags, it will give you as a pattern in the hemoglobin FAB, FA Bart's.

Next. This is a typical picture of the BioRad HPLC just for those who did not know what a HPLC looks like.

Next. This is a typical picture of our IEF. The components of all the IEF, the power source, the chamber, and of course the water coolant that we use.

Next. I wanted to show what the peaks looks like and in Tim's presentation, we saw that while he was explaining the different molecular aspects on how these peaks are derived. But you can see, first of all, the pattern that is given for the HPLC is it would just indicate FA Bart's, flagging you that there is a Bart's so you can look at. Then you can check the percentage of the Bart's that is in there. If it really amounts to that level where your state accepts, which is 25 or 20 or 30 depending on what the state has defined, then you'll have to act on that further. In IEF, the red arrow is an indication of what it is as a fast band that we see. In HPLC, we see fast Bart's there and in IEF, we look at it as fast Bart's.

Next. Again, this is another sample in a different form that we have seen how these things go. But one of the things that has to be very clear is that Tim has explained the different levels of Bart's, the normal, the silent, the carriers, and of course the disease. One of the things that we must put in mind is that sometimes in the middle of a summer like this, we can find a lot of fast bands or even Bart's in testing. We have to be very careful that it is what the percentage because hemoglobin degradation can show up as fast bands as we see with this red arrow, but you need to know the strength of the bands or the percentage in HPLC.

I think that concludes my algorithm [inaudible 00:30:29].

Tim Davis: Great. Thank you, Joseph. Our next speaker is Christine Dorley. Chris completed her undergraduate studies in medical technology from Western Kentucky University in Bolling Green, Kentucky and obtained a master's degree in public service management from Cumberland University in Lebanon, Tennessee. Christine is currently the newborn screening division manager for the state of Tennessee. In this position, she oversees testing of newborns for metabolic and genetic disease, including hemoglobinopathies. Welcome, Chris.
Christine D: Thank you, Tim. Thank you everybody for dialing in today. I'm going to be talking about results from a survey on alpha thalassemia that was produced by the hemoglobinopathy workgroup that's comprised of several state laboratories, representatives from the Association of Public Health labs as well as the National Center for Blood Disorders there at CDC. As a workgroup, our functionality is to set capabilities for the state laboratories. Can you go to the next slide, please?

We really wanted to learn what states were doing with regard to alpha thalassemias so our survey's purpose was to gather information that would inform us so that we could design a training webinar. For example, what we have already covered in part one and then for today, part two. Also, to make recommendations on how to report hemoglobin H disease and other alpha thalassemias. We really, really wanted to highlight areas of improvement in reporting alpha thalassemia. Our survey was sent to 53 newborn screening programs in October 2016. It was communicated to laboratory directors, lab managers, and follow-up coordinators and personnel. We were surprised. 41 programs responded, which makes it around a 77% response rate. We ended up having to exclude programs that responded with answers as I do not know or that they outsource their testing to contract laboratories.

Let's dig into the survey. Can we go to the next slide? We'll start with question number two because question number one was to ascertain the state's identity and we want to present this data as aggregate data. We'll start with question number two. We understood that some states performed only one method of screening. Joe, in the earlier presentation, alluded to the screening algorithm. We really wanted to know what people were actually doing. Were they only doing one method? Were they doing two methods? We asked, what is the hemoglobin screening method and algorithm used by your program? 63% reported that they use two screening methods, whereas 37% only reported one. For the states reporting only one method, we wanted to know what was the method. Well the majority used isoelectric focusing compared to HPLC.

Now in the next slide, for those states that were using two different methods, the majority of the states indicated that they use IEF compared to HPLC. Then for the second method the majority sued HPLC followed by IEF. It seems to us that the most popular screening algorithm is IEF first, then followed by HPLC. Some states flex to molecular testing. Then others indicated methods that were not named per se. Based on this information, unknowingly, we really wanted to get a feel for what and how people were reporting hemoglobin Bart's for those who were actually testing for it.

Let's go to the next slide. We asked, does your newborn screening program report hemoglobin Bart's finding? Surprisingly, 95% of the participants indicated that they did. For those you would potentially answer no, we wanted to know the reasons why and to give us some comments. Those 5% that answered no, they said that they don't follow-up for hemoglobin Bart's. Others said that they couldn't confirm for it or quantify it so it was not an issue for them.
another one indicated that they didn't screen for alpha thalassemia because it didn't appear on the recommended uniform screening panel. For those people who answered that they did reports Bart's findings, we wanted to delve into how they actually reported alpha thalassemias so let's go to the next slide.

We asked, what types of alpha thalassemia were reported? Well some states said they reported hemoglobin H, alpha thal trait. Some said they reported silent carriers and then others indicated that they also reported thalassemia major. But the majority of people were reporting alpha thal through reporting Bart's. 30% of states chose that they reported by other means. We were wondering what this meant. For those that did state other, we wanted to know what the meaning of that was. They commented that they couldn't differentiate the types of Bart's so they only reported Bart's. One state commented that they report only sickle beta thalassemia and if they saw bands that were in combination with FA or FAS that was Bart's, they would report that. One state reported that while they could report all of the categories, it was a possibility that they could miss some alpha thalassemia because of changing cutoffs or because of reagent lot changes. Then another state indicated that in actuality they could report alpha thalassemia major, but they never had an opportunity to because they never identified an infant with it. We also wanted to know for individuals who were reporting Bart's, to whom did they give the reports to. We'll talk about that on the next slide.

Here you can see some percentages of the distribution for reporting to whom. The majority of participants reported that the results were communicated to the physician of record and also to the newborn screening follow-up program. A few states reported results to parents and a few also communicated to other providers. We wanted to know in case someone did answer as other, who defined other.

On the next slide, we learned based on comments from those reporting as other, that they reported to the birthing hospital. Some reported results directly to the hematologist. Some reported to the regional sickle cell center or special care center, their results. Some reported to specialists with whom they have contract for care of these infants. Some reported directly to the PCP as well as the hematologist if the demographics stated that the infant was of Asian descent. Interestingly, we wanted to know what percentage cutoff people used for reporting Bart's or for the different types of alpha thalassemia. We'll talk about that in the next slide.

Question 5, what are the criteria or percentages used for reporting the different types of alpha thalassemia? For alpha thalassemia trait, there was an average cutoff of around 9%. Some people report alpha thalassemia trait as low as 2% hemoglobin Bart's, whereas others reported as high as 20% or greater. Then for alpha thalassemia major, the average was 64%. Some people reported alpha thal with a minimum of 25%, whereas 100% would be indicative of alpha thalassemia. For hemoglobin H disease, the average was roughly around 21%. Some people reported hemoglobin H disease as low as 11%. Others it had to be
as high as 31% before it would be reported as hemoglobin H. Then some people, three participants, did report for silent carriers. An average percentage of 7% with a minimum of 3 and a maximum of 11%. But this information didn’t cover constant spring necessarily so we wanted to know what states were doing for constant spring.

The next slide asked basically concerning constant spring, does your newborn screening report constant spring? 92% of participants said no, that they did not report constant spring, whereas 8% said yes. We wanted to gauge for those who were reporting constant spring, how they confirmed it. Well one indicated that they detect a constant spring through the presence of gamma chains that were detected through their screening algorithm as an undetermined variant and this undermined variant would then subsequently require confirmation at a hematology center. Others said that they used a reference laboratory using DNA testing for confirmation. Others mention deletion specific PCR, which would analyze the globin gene for the more common mutation.

We also were curious to know if states had any kind of recommendations on their mailers for retesting or follow-up. In this next slide, for question number seven, we asked, does your newborn screening provide recommendations for patient retesting or follow-up? We also wanted to know for the states that answered yes, that they gave recommendations, what these recommendations were. Well 11% said no, they didn’t recommend anything. They only reported, whereas 89% said that they gave recommendations. Some of these recommendations included genetic counseling, referral to pediatric hematologists. Others recommended testing with some other assay that was more conclusive. Others asked for a repeat newborn screen based on what percentage of Bart’s was reported. They also recommended having a complete blood count or a CBC between nine months and twelve months of age. Others recommended carrier testing as well as genetic counseling also for the family.

We also wanted to know what sort of educational resources were out there for labs to turn to regarding alpha thalassemias. On the next slide, we asked for the states to share any materials or website links that they found regarding alpha thalassemia that they use in their program. We had quite a few people to share. About ten states provided website information. Some of these were linked to [inaudible 00:41:30]. Two listed organizations as resources, such as the National Newborn Screening Global Resource Center. Others gave us copies of fact sheets as well as decision trees used for reporting. Then others shared publications from related to alpha thalassemia. Let’s go to the next slide to see some of these.

There in the top left corner is a publication by Virgil Fairbanks, editor from 1980, entitled Hemoglobinopathies and Thalassemias Laboratory Methods in Clinical Cases. In the bottom left corner, you have a publication for parents produced by the California Department of Health Services for Hemoglobin H Disease. Then on the right is a reference out of Harvard University that’s a tutorial basically on all things thalassemia, which is actually a really good thing to take.
Next slide shows publications surrounding alpha thalassemia. For example, the one by Vichensky there, 2013 Clinical Manifestations of Alpha Thalassemia. You have the evidence review that was presented to the RUSP in 2010 for decision to add alpha thalassemia screening. Then there in the upper right corner, another article on hemoglobin H disease about the clinical course and disease modifiers. Then a publication in 2010 by Alex Kemper called, Weighing the Evidence for Newborn Screening. You can see there is some public information out there, but it's not a lot.

Now considering our survey results as a workgroup, what did we learn and conclude? Next slide, please. As a whole, the majority of states that answered our survey are screening and reporting alpha thalassemia, which is a good thing. To get the remaining states, the 5% that aren't screening and reporting, would be a marked improvement. The survey, in my opinion, I think met its purpose. That was one, to educate about thalassemia. Secondly, to highlight the importance of screening to detect disease. Then also through the webinar, part one and part two today, we were able to bring awareness to states that alpha thalassemia is really more common that what was previously thought in our population. With that, I'm going to end here and then turn it back over to Tim for questions. Thank you.

Tim Davis: Thanks, Chris. During this question and answer session, you can press *7 to unmute and ask questions over the phone or type your question into the chat box. Oh, it says it right up there.

Careema Yusuf: Thank you, everyone. Does anybody have any questions for our presenters? You can press *7 to unmute your line or if you'd like, you can type your question in the chat box and I'm happy to read that out.

Somebody had a question in the chat box. They were asking if the presentation will be available following this call and the answer is yes. In a couple of days, we will post the website to the APHL Newborn Screening and Genetics Training webpage. On there you'll find this recording as well as a transcript for the webinar.

We do have a question in the chat box. It says, has anyone had experience with homozygous constant spring?

Tim Davis: Yeah. I can answer that. The problem with homozygous constant spring is if you're using PCR allelic discrimination, the other alpha two might be deleted. If that's the case, then it'll look like it's homozygous on your report, but it's actually deleted. It's difficult to tell. There's been a few where the bands have been a lot stronger, where we've suspected that it's homozygous constant spring, but for us in Washington, we can't distinguish that.

Careema Yusuf: Great. Thank you, Tim. Does anyone have any questions that they'd like to use the phone line for? You can press *7 to unmute.
Okay. I do have a couple questions in the chat box. One asks, if during the heated months, according to Joe, the bands show up for Bart's, do these same bands show up on HPLC?

Joseph Ubalke: Yes. They do show up on HPLC, however the percentage is very small.

Careema Yusuf: Okay. Thank you. Any questions on the phone? You can press *7 to unmute.

I do have a question in the chat box. Any thoughts on what type of evidence may be needed for alpha thalassemia to be included on the RUSP? Based on the feedback received in 2010, it looks like more data may need to be collected.

Tim Davis: I think that's kind of been the real problem is there hasn't been a lot of clinical data out there on these [inaudible 00:47:06]. That's kind of why I had Bender and Dr. Aguinaga speak in the last session. It's still a little anecdotal, but for hemoglobin H constant spring, it seems to be pretty persuasive. I think we just need to have more data and we're still waiting on that so that's where we're at.

Careema Yusuf: Thanks, Tim. Anybody have any questions to ask on the ...

Dr. Aguinaga: I had a comment.

Careema Yusuf: Sure. Please go ahead.

Dr. Aguinaga: Yeah. To the person who asked about homozygous constant spring, it's likely you will see 5% constant spring peaked on the HPLC. If it is hetero, you're probably going to see 2% or so.

Careema Yusuf: Thank you, Dr. Aguinaga. Any questions on the phone? If not ... Somebody's asking, is there recommendation that states should adopt a two screening method?

Tim Davis: Yeah. Actually, I would say yes. I think that having two different chemistries when you're doing hemoglobinopathies, having two different chemistries to finalize your result is always a good thing. The more methods you include, I think you get better results so I would say yes.

Joseph Ubalke: In my own personal experience, the same answer is yes. Why? If you're doing IEF only, if you're looking for A that is very low in the IEF. It's so faint. Sometimes it looks like it's not even there. But in HPLC, it will give you the number that you know there is A there, even though it is 2% or 3%. With 2%, if you do IEF, you may not even see it in the IEF.

Careema Yusuf: Thank you. I do have another question in the chat box. It says, great informational webinar. Is it time to re-examine hemoglobin H for the RUSP?
Tim Davis: I would say yes. Again, I’m going to talk at the symposium so I think we’ll have a little bit more information on that. I’m not sure who the person out there is going to be that’s going to do this again. I don't know if Vichensky can do this again or if he wants to. But hopefully there will be some push for it. I think we need it.

Careema Yusuf: Great. Thank you. Another question is, how accurate is the percentage of Bart's on BioRad’s HPLC. Are there any interfering substances that cause an elevation of the fast band?

Tim Davis: I think it's very accurate. From everything that we can tell, from our results. And again, here in Washington, we actually use two HPLC systems at one time so we were able to get pretty good correlation. One of them had really good resolution. There was nothing interfering that we could tell that was interfering. But again, we're not running a tandem mass spectrometry on these so we don’t know for sure everything in the fast region. But for everything we can tell, it's pretty stable, at least in the newborn period. I would say no, that there's not a lot of interfering. Now what does happen is that eventually hemoglobin H is going to form so there's going to be more bands and more peaks in that region. But in a newborn, it's pretty well-established.

Christine D: This is Chris. I'll second what Tim said. As a BioRad HPLC user here at our laboratory, it's pretty consistent reporting for hemoglobin H. We even had hydrops fetalis that we reported that was confirmed so it's pretty sensitive and specific. That's my opinion. I'm saying that it is.

Careema Yusuf: Thank you. Tim, could you please clarify the symposium? I don't think folks know what that is. If you could just clarify what you mean.

Tim Davis: Yeah. During the RUSP portion, which is the first day of the symposium, I'm going to be speaking ... I'm going to be doing a 15 minute presentation so I'm going to be talking about alpha thalassemia there. I think I'm just going to add more information than what we currently have right now. I'm going to try to actually reach out to Hersa and get more information about basically defining the relationship between hemoglobin and the RUSP. It's a secondary condition right now and there's a lot of other hemoglobinopathies that definitely beta thalassemia major is one that should be there. Were finding it as a secondary condition so I’d like to kind of explain more about the relationship of the RUSP to hemoglobinopathies so that's what I'm going to talk about.

Careema Yusuf: Thank you. Just to add to that, the symposium is the APHL Newborn Screening and Genetics and Testing symposium that will be held September 10th through the 13th of this year in New Orleans, Louisiana. If you’d like some more information about that symposium, please feel free to email me and I can send you that information.
Christine D: Also Careema, if I'm not mistaken, at the symposium, there may be a round table on alpha thalassemia so everybody can come and kind of we can fire up some discussions about what needs to happen in the future.

Careema Yusuf: Great. Any questions on the phone? We did have a clarification question in the chat box. I think it was the question about a two screening method and the person wanted to know if the question was about two newborn screens or two methods on the one screen. I believe it was the latter. They were asking about two methods on the one screen.

Tim Davis: Yes.

Careema Yusuf: Okay, great. Does anyone have any questions? You can press *7 to unmute or you can write your question in the chat box.

Okay. Hearing none, Tim, would you like to close out, please?

Tim Davis: Sure. A recording of this webinar will be archived on the APHL newborn screening and training webpage. You will receive a link to this page in your post-webinar follow-up email. PACE credits will also be awarded for attending this webinar. To receive PACE credits, you must complete the post-survey evaluation, which will appear in the post-webinar popup window and follow-up email. If anyone has questions, you can contact Careema Yusuf and she has her information there on the slide. Thank you very much for joining us and take care.

Careema Yusuf: Thank you.