APPLYING MYCOBACTERIUM TUBERCULOSIS GENO
August 24, 2011

David Ashkin: So without further ado I want to turn it over to Wendy. Wendy are you there?

Wendy Cronin: I am there.

David Ashkin: How are you doing?

Wendy Cronin: I'm fine, how are you David?

David Ashkin: Good, now Wendy you know yesterday we had a really weird situation, while we were all preparing for a hurricane, you had the audacity to take away the headline and you guys suffered from an earthquake. What happened, what did you do?

Wendy Cronin: I stamped my feet one time too many.

David Ashkin: How is everything up there, everything going good?

Wendy Cronin: Fine, yes, we have no damage but it sounds like there's some damage at the Washington Monument and the Capitol I think so it was pretty impressive, most of us had not experienced a hurricane in Maryland.

David Ashkin: Well Wendy you know what, you know we really, really want to thank you for actually despite everything going ahead with this program and without further ado Wendy I turn it over to you.

Wendy Cronin: Okay, I'm just watching for my slides to come up. Can you hear me better? Okay so the first thing that I'm showing you is that we have a baby and that baby was culture positive with tuberculosis.

Donna I'm not able to - oh there we go, okay. Sorry, I'm just getting started on a different computer than I practiced on.

So there was a contact investigation, a source case contact investigation around that baby and in that investigation identified a babysitter with TB who had been infectious for quite some time and also a toddler with culture positive TB.

And all this occurred before genotyping results. Next slide, next slide please. After genotyping, we found that the three cases, initial cases, the baby, the toddler and the babysitter all had the same isolet genotype.

But there was another case with the same isolet genotype who turned out to be an assistant minister. And when we talked to him it turned out that the babysitter was also a church deacon in the same church and had transmitted TB to that minister.

Next slide. The contact investigation around the assistant minister identified another minister but it also identified that our babysitter, church deacon had actually been the person who infected the second minister.

So this is mixing up contact investigations and genotyping information. So can we go to the next slide? After genotyping, it turned out that there was another case who had the same genotype and that case was a hotel chef.

And investigation of that hotel chef revealed that our babysitter was also an assistant cook. Next slide. So this slide shows the combination of people in this small outbreak that we had that was caused by highly infectious source case.

The blue arrows identify the transmission that occurred and was identified through contact investigation but the red arrows were all demonstrating transmission from the babysitter, also church deacon and assistant cook to ministers in his church and also hotel chef where he worked.

So we now have three settings, the babysitting setting, the church and the hotel kitchen and two of those settings were only identified after genotyping occurred.

So I wanted to just use this to whet your appetite and now I'll turn the presentation over to Lauren to describe exactly what TB genotyping is and how it's done.

Lauren Cowan: Good morning. Please let me also extend my appreciation for your attendance today. I hope that Wendy has piqued your interest in learning more about genotyping.

I know that she has many more fascinating examples to share with you later in the presentation. But first she wanted me to take a few minutes to provide you with an overview of genotyping.

So we'll start at the beginning, why do we genotype mycobacterium tuberculosis? Well we use genotyping to compare isolets and to answer the question are they the same or are they different?

Now this is a relatively simple direct question and one that small children enjoy answering, are these two pictures the same or are they different?

In this example the picture shows a dog or a cat and so we conclude that the pictures are different. In this second example the pictures contain the same elements and so we conclude that the pictures are the same.

So we use genotyping in the same manner to create a picture if you will of the mycobacterium tuberculosis genome. And then we compare these pictures to determine if the two strains are the same or different.

So hopefully now you understand the basics of genotyping. And we need to explore two assumptions that we make while we are interpreting these genotype pictures.

Our first assumption is that there are so many different strains of mycobacterium tuberculosis circulating in a community that the best explanation for two patients being infected with the same strain is that the two patients share some type of relationship.

The diversity of strains circulating in the United States is quite high, through the activities of the National TB Genotyping Service we have detected more than 13,000 different strains and identify new ones daily.

In this picture I reflect the strain diversity in a community using colors. Notice that only two patients, I don't think my animation is quite working, have the same color pink genotype strain.

Our assumption would be - there it is - our assumption would be that the best explanation for these two patients being infected with the pink bacteria is that there must be some type of relationship between these two patients.

This relationship could be as simple as direct transmission shown here or more complex as Wendy will demonstrate later.

The relationship could be relatively recent or quite long ago. The diversity of TB strains in the United States reflects the diversity of its TB cases and in a community such as the Philippines where the introduction of new strains is limited, the diversity of TB strains can be much lower.

This decreased diversity greatly limits the ability to infer relationships between patients. Our second assumption is that the genotyping method being used characterizes regions of the genome that change frequently enough to create diversity but not so frequently that it changes during a chain of transmission.

For example if the genome changed so rapidly that it changed from pink to blue to yellow to green while being transmitted, I admit this is somewhat a ridiculous example, but it proves its point, we would not be able to use genotyping to infer any type of relationship.

Likewise if the genome never changed we would have no strain diversity and again no ability to use genotyping to infer relationships.

Our picture of the genome needs to include elements that change not too slow and not too fast but at just the right rate.

To create our pictures of the TB genome, we use two or three different genotyping methods to characterize small segments of the TB genome.

Our picture is very incomplete as these segments encompass only 1% of the complete genome. I'll introduce these three methods to you in a moment but first let me answer the question of why we use multiple methods.

Do you remember our pictures from the beginning? Each had three elements, a building, a tree and a pet. If we only compared our pictures based on two elements we might conclude that the pictures are the same when in reality, they are different.

We use multiple genotyping methods to provide as much information as possible. All elements equally make the pictures different, it doesn't matter if the pictures contain both the same dog or the same dog and house or the same house and tree, if any element is different then the pictures are different.

Likewise, if two strains have the same MIRU-VNTR pattern in different spoligotypes they are different. And if they have same spoligotypes and different MIRU-VNTR patterns they are still different.

And even though we use multiple methods to increase the information presented in our pictures of the genome, in the end we are really only looking at 1% of the picture.

Sometimes that small picture will provide you with sufficient evidence to conclude the two strains are different. But other times the pictures can appear the same when in reality they are different.

Matching pictures can be used as evidence to support the existence of a relationship, but never to prove that relationship. And genotyping, it's important to remember that it's always easier to demonstrate that two strains are different than it is to show that two strains are the same.

Now let's turn to our methods. Spoligotyping examines the variation found at a single locus or location in the genome. This direct repeat locus contains 36 base pair directly repeated sequences separated by short spacer DNA sequences.

The assay is designed to detect the presence or absence of 43 of these short spacers. Here we have the 43 spacers in a line with black boxes indicating that the spacer is present in this strain and empty spaces indicating that the spacer is absent.

We create a 43 digit binary result for the spoligotype by indicating the presence of a spacer with a one and the absence with a zero. Now if you've seen a spoligotype and a genotype report you know that it doesn't contain any 43 digit binary results and you are correct.

To make the spoligotype result more compact we report the octal designation for this number and that results in the 15 digit result that is contained on your genotype reports.

An important thing to note here is that these are actual results and not a type designation name. MIRU-VNTR typing differs from spoligotyping in that it

examines variations found in multiple loci or locations distributed throughout the genome.

These loci contain tandemly repeated DNA elements and a number of those repeated elements differ from strain to strain.

We analyze each of these loci individually to determine the number of tandem repeats present and then concatenate these results into a string to generate the MIRU results.

To keep the strings at 12 characters we use the convention that an A is ten copies, B for 11 copies and so forth. A dash in the results indicates that no result was obtained for that locus.

When the National TB Genotyping Service was launched in 2004 we characterized 12 MIRU VNTR loci, and in 2009 we expanded that assay to provide even more information to include an additional 12 VNTR loci.

The original 12 we report to you as MIRU1 and the new 12 are report as MIRU2. Spoligotyping and MIRU-VNTR typing are routinely performed on all isolets submitted for genotyping to the National TB Genotyping Service.

A third method that is available upon request is IS6110-RSLP fingerprinting. This assay is based on the variation found between strains in both the number of copies of IS6110 and their positions in the genome.

Unlike the digital results generated by spoligotyping and MIRU-VNTR IS6110 results are pictorial and thus more difficult to report and compare.

IS6110 fingerprints are named as they are identified. This method can be used to add additional information to the picture of the genome.

Now you have a chance to be involved. Let's look at some genotyping results. Do you think that these results suggest that there is a relationship between these two patients?

I'll give you a chance to look at the picture and then we'll move on. And here is where you can answer. And it looks like 94% of the people say false and they are correct.

These results are completely different and that would suggest that there is no relationship between the two patients with these isolets.

A second example, let's look at these genotype results, could there be a relationship between these two patients? And overwhelmingly everybody think it's true and yes, the results are the same and there very well may be a relationship between these two patients.

Looking at the same results that we just looked at, do they prove that there is a relationship between these two patients? And the result is not quite as clear cut, but no the results are consistent with the conclusion that there is a relationship but they do not prove that conclusion.

Now let's look at two very similar genotyping results. Notice that they are nearly identical and differ only at one locus in the MIRU1 results. Do you think these results suggest that there may be a relationship between these two patients?

Again not quite as clear cut an answer, and I have to apologize for this because it was kind of a trick question. An analogy with our pictures would be that the apple tree lost one of its apples, we really don't know when the apple fell off the tree.

It could have been yesterday or forever ago. In genotyping, we call these really, really closely related genotypes one off.

We don't use these one offs results to conclude that the strains are different or the same. Both answers are correct. The change could have happened long ago and there is no relationship between these two patients.

And the change could have happened more recently and there could be a relationship between these two patients. In other words, these genotype results are consistent with both answers.

So everybody got it right. Our last example is the genotype results of genotype G, contains a dash indicating no result was obtained for that locus.

But it matches that of isolet H in all other regards. Do these results suggest that the isolets are different?

It looks like everyone's voted, that's right, the answer is false. There is no data here that can be used to show that the isolets are different.

Finally let me introduce you to some of the nomenclature we use for TB genotyping. First of all as each combination of spoligotype and MIRU1 pattern is identified at the national level, it receives a PCR type name.

Next as two PCR types are identified within a state it receives a state cluster name. PCR types are comparable between states. State cluster names are not.

These naming conventions were put into place prior to the expansion of MIRU-VNTR to 24 loci to incorporate the new results into the state cluster name as two isolates within a state have the same spoligotype, MIRU1 and MIRU2 results.

As soon as that is identified a state cluster name two is defined. These state cluster name two's sub cluster within the original state clusters to provide you with continuity with results obtained prior to 2009.

The National TB Genotyping Service is funded by CDC and provides for the genotyping of one isolet from every culture positive TB case in the United States. Since 2003 over 70,000 isolets have been genotyped.

I cannot take the credit for this enormous undertaking and would like to take this opportunity to acknowledge the work done by the genotyping labs located at the Michigan Department of Community Health and the California Department of Health Services.

I'll be happy to answer any question you may have at the end of the presentation, but now I'm going to hand the microphone back \over to Wendy to present how genotyping can be used in programmatic activities.

Wendy Cronin: So I'm going to give you my presentation objectives about the application of genotyping. Well if they'll show, there's - okay one is to interpret the meaning of a genotyping cluster. Next is to describe six ways that TB genotyping can be applied to local programs.

Next is to describe steps to conduct a cluster investigation and to describe how genotyping can be used to evaluate and prove local TB control activities.

So I have a question for you, does genotype clustering of isolets from different patients indicate recent transmission has occurred?

We got a nice mix of answers here, and the answer is yes sometimes. It indicates the recent transmission may have occurred but you need more information in the field to understand that answer.

Why is genotyping so important to us? Genotyping helps us in a number of ways in our local programs. One is to identify and interrupt recent transmission to prevent outbreaks or to slow down outbreaks or stop outbreaks earlier than they would have stopped if they had been allowed to continue without intervention.

It helps us to identify unsuspected relationships and it enhances contact investigations because we identify previously unidentified source cases as I showed you with the babysitter and the church and the babysitter and the hotel restaurant.

And it helps us identify previously unidentified locations. You can use genotyping to distinguish relapse from new infection of a person, you can recognize false positives.

And you can monitor trends and evaluate TB program performance. So a genotype cluster as Lauren said is when an isolet genotype matches at least one other person’s isolet genotype and in this example you can see that the spoligotypes and the 24 loci for MIRU match identically.

What does the cluster mean, does the isolet match at least one other isolet in the database? The answer is yes, it depends on your database however, you might be looking at a state database, a large metropolitan area database or a national database.

And it may indicate recent transmission but not necessarily, and we will talk about that in a minute. If the isolet doesn't match another isolet in the database we call this unique.

And this could mean a number of things, it could indicate reactivation of an old infection, if someone got infected many years ago the organism may have changed enough that we identify a unique isolet with their past infection but not with recent TB infection.

It may indicate imported TB which means TB came from people that came into the country, they were infected in their country of origin and they brought their organism in with them.

But unique can also result from missing isolets so if 60% of the isolets from your culture positive patients have been genotyped and you have a unique pattern for a patient, you don't know whether there might have been a cluster with one of the 40% of isolets that weren't submitted for genotyping so it's really important to have high proportions of isolets submitted for genotyping.

I believe now the national proportion is over 80% and certainly if you can get up to 85, 90 or 95% you have good clustering data.

So if two cases are clustered genotypically is there true evidence of ongoing transmission? Well the genotype laboratory information that Lauren so eloquently described is only half the picture.

To determine whether recent transmission actually occurred both genotyping and epidemiologic information is needed and this is what George Comstock used to call shoe leather epidemiology.

And in that case we use epidemiologic links or what's commonly known as an epi link which is an identified relationship between TB patients.

So in this case we have a small child who goes to daycare who has active TB and a positive culture. And that child's isolet matches the isolet from the daycare teacher.

So probably this link might have been found out through contact investigation because the child investigation, the source case investigation around the child might have been conducted.

But it turns out in our database that there's this very dashing, debonair young bartender who also matches the other two patients but we have no link, nothing was identified during contact investigation.

When we went back and talked to that debonair bartender it turns out that he dated the teacher at the daycare center for a short time during her infectious period, but because that was some time in the past, she had forgotten to mention it when she was being interviewed for her contact investigation.

So epi links are essential for determining ongoing transmission and to get at them we want to know personal characteristics such as demographics and risk, homelessness or substance abuse.

And we want to know the place where the people were, where they located at a place where they spent time together. These could be bars, jails, homeless shelters, churches, they could be in the same apartment building.

Or they could maybe play cards together or hang out together or they could be of course in a household, work or school.

And we also need time, were they together at the time that one of the two patients was infectious? So I want to give you a tale of two investigations.

A contact investigation differs from a cluster investigation in that a contact investigation centers around one TB case or suspect.

A cluster investigation takes place after genotyping information has been received from the genotyping lab and it involves two or more cases that have the same genotype and therefore are in a cluster.

The contact investigation is centered around named contacts. A cluster investigation is centered around finding epi links between cases that have the same isolet genotype. Contact investigation occurs when the infectious case or suspect is identified but the cluster investigation doesn't occur until after the report's received.

Contact investigations generally take place in households and they may spread to workplace, schools or congregate settings. But cluster investigations also identify unusual settings like bars, religious gatherings, card games and other settings that might not be picked up in a routine contact investigation.

Contact investigations depend on interviewing the case in suspect, doing record reviews and sometimes site visits and cluster investigations are similar in that you review the genotyping data first.

But then you interview the case manager to find out what they know about the case or suspect or the two cases that have the - they're not case suspects, the two cases that have the same patterns.

We also review contact investigation records and case medical records because sometimes even if the cases didn't know they were on the same place, perhaps they were in a hospital at the same time.

This might be mentioned in their medical record. And then we go out and reinterview those cases if we can't find the link any other way to identify whether they knew each other or spent time in the same place.

And we also conduct site visits in cluster investigations. The purpose of the contact investigation is to identify patients with latent TB infection or newly identified patients with TB.

And of course the purpose is to prevent new cases of active TB. Contact cluster investigations are very similar, we also want to identify new cases with infection or TB and prevent new active TB.

One of the ways we do this is we make a decision about whether we need to expand the contact investigation. If we had expanded the contact investigation around the babysitter that I described in the introduction, we may have discovered that that person also was an active member of a church and worked in a hotel kitchen.

And we may have identified those links. So these are data from Maryland about the proportion of epi links that were detected by routine contact investigation and the epi links that were detected only after contact investigation and genotyping results were received.

So about two-thirds of epi links were identified through routine contact investigation but 37%, almost 40% of the links were only identified after we investigated when we got the genotyping information back.

So I'd like to ask you what locations do you think the routine contact investigation missed? This is kind of fun to watch. Those of you that voted for social settings were correct.

Households it's possible they could miss a household, so the people who voted for household school and workplace certainly contact investigations can miss those places.

But the place that genotyping has really identified are social setting that we need to ask more about where people spend their time outside of household, schools and workplaces.

And so this is what we've discovered and what we're working on, to try to improve the way contact investigations are conducted.

To identify epi links as I said before we talk to the health department. We talk to the case manager for the cases who are in the cluster to find out if they already knew about a link and then if they do then we document that and we don't do any more on the cluster investigation.

If the case managers don't know, we like to look in the medical records and the contact investigation logs, maybe one of them named each other in a contact investigation.

Maybe a place was common in the medical records and we try not to interview patients again if possible, but we do this if necessary because we want to understand what's happening with transmission, that's the only way that we can actually intervene.

So what do we look for? The one case named each other as a contact, did the cases name the same contact? Maybe there's a common person in between that suggests a relationship between our two cases.

Do they live, work or spend time in the same place? The infectious period is very important and if they're foreign born we want to know what the country of origin is.

Was the other person in the cluster from that same country, do they socialize together, do they both bring that organism in from their country of origin?

And when did the people arrive in the United States? If the second case in a cluster arrived right before diagnosis it's unlikely that they were infected by the first person in the cluster because they wouldn't have been in the country long enough.

They probably were already infected and had active disease at the time they arrived. So these are all things we try to do to understand what really happened.

So this is another outbreak I want to go through briefly. The diagonal ones were patients that were linked only through genotyping and the solid circles are patients who were identified through genotyping and also through contact investigation.

So we have a worker one, this worker had a person who came and worked on her house and so the painter wasn't identified because normally you don't think of a painter who paints the outside of your house as being a person who's likely to transmit TB. But it turned out once we investigated these two that the - there was a relationship between the worker and the painter.

And the painter spent quite a bit of time inside the house as well as outside. And we also knew that this worker had infected several other workers in the same workplace which turned out to be a fishery house.

What we didn't know was the worker one was also a cook in a restaurant, or she was a waitress in a restaurant and a cook in that restaurant also became infected, this was identified after genotyping.

Another worker who worked in another fishery in the same area was identified through genotyping and it turned out that that worker drank a lot with worker number two.

Worker number three had a girlfriend who was infected and another person was identified and it turned out that that person rode in the same car every Sunday to church with the girlfriend.

And then we also identified a brother who was in daycare. Fortunately there was no more spread in that daycare and a grandchild and we had a 12th case who we never were able to link back to the cluster but we're quite certain

because this was the only person without a link that something happened somewhere in this cluster but we were just unable to identify that relationship.

So epi links are commonly divided into known links, possible links and no identified links. And sometimes with known we also talk about probable links.

So no link is when patients name each other perhaps as contacts or during the cluster investigation or they were in the same place at the same time which could be a homeless shelter, it could be a church.

A possible link is when patients spend time in the same place around the same time but we couldn't get the overlapping time - overlap of time proven. Sometimes this happens if they're in the same neighborhood.

But we couldn't actually prove that they were spending time together or they were in the same homeless shelter but the dates weren't really clear and it could also be a geographic area.

We also talk about recently nearest neighbor and GIS analysis, what if they just live in an area really close to another person or if they share social and behavioral traits at the same time that they were in that area like substance abuse or homelessness.

All these things increase the likelihood that a link exists. We never say that there's no link, we always say there was no identified link because it may just be that somebody doesn't know the name of another person or they're not willing to talk about relationships that they have.

So we're never sure whether there's not a link but we know sometimes we can't identify a link. The next thing that's really important is to understand

what happened in transmission, so we want to determine the probable source and secondary cases.

And if you work by the date of diagnosis the cluster looks like it falls out this way, patient number one infected patient number two, patient number two infected patients three and four.

But if we work from the time of symptom onset which is not something that we collect nationally for good reason, it's not a very reliable date, but if we find out and talk to a patient about when they actually started coughing or when they showed symptoms, we may get another picture.

And in this case it turned out that patient number two was the source for patients one, three and four. And in our large outbreaks it usually works out this way.

The index patient who is patient number one is different from the source patient. The source patient may have been infectious sometimes we found for six months or even a year.

They're coughing, they keep going to emergency rooms, TB is not diagnosed. Meanwhile their secondary cases are already being identified before the source case is actually identified and diagnosed as a TB case.

So watch out for this kind of scenario, especially when you have outbreaks that involve five or more people.

So the definition of an infectious period is the time when a person is capable of transmitting TB to others who share the same airspace and it's usually estimated by the patient reported date of onset if you can get that.

And of course the patient to transmit is 99.999% of the time pulmonary. So this is adapted from the CDC chart on estimating the infectious period for contact investigations.

In our case the patients need to be sputum culture positive, they don't have to be smear positive. We know that 20% of transmission occurs from smear negative people who are sputum culture positive.

They may or may not have symptoms, they may or may not be curatorial, though these are all indicators of level of severity of disease and perhaps increased infectiousness.

And so we say the beginning of the infectious period is three months before symptom onset with a first positive finding consistent with TB which could be the chest x-ray, the date the sputum was collected for smear, the data was collected for culture, whichever is longer.

So I have another question for you. MTB genotyping has enhanced TB contact investigations by demonstrating what?

Very good, contact investigation interviews need to question about social settings and locations. And we're working on creating a form that will do just that that may increase contact investigations.

So in summary recent transmission is a search for commonalities. We look for information common in the cases, demographics and risk factors. We look at infectious periods, we want to know work and school histories, social history is very important.

Travel history could be important, history of TB exposures, somebody might have broken down from TB from an exposure long ago, just because they're in a cluster doesn't necessarily mean they had recent transmission.

We always want to look at contact lists and of course location, location, location is essential for these investigations.

The next thing I want to talk is relapse or exogenous reinfection. Now this is a fake homeless person. But given the idea that we had a homeless outbreak in Baltimore that was well known by a particular pattern, we have this new person show up, Rodney Homes who's homeless.

And so we all say yes, he's going to be part of that homeless outbreak that we've been dealing with. But when we got the genotype results back on Rodney Homes, it turned out that the spoligotype and MIRU were different.

And he was in a different cluster. And so that lead us to go back and look at prior genotyping data and it turned out that four years earlier we had an isolet genotype from Rodney Homes and he had the same pattern.

So this told us that he was not infected by the homeless outbreak strain but in fact he had relapse of his earlier infection. And we do see this in the states, most of the time when a patient has TB a second time it's actually due to relapse.

In developing countries where there's a high prevalence of TB and lots of likelihood of exposure it's just as likely that the person was infected by a new organism as if they had relapse of an organism which they already had.

Next I want to talk about false positive cultures. So what happens with a false positive culture? Whoops, went too fast. Usually something will happen in the laboratory where two test tubes touch each other, there's some aerosolization often invisible that occurs so that the organism spreads or sometimes there's a spill.

Often when we investigate false positive cultures we find it very hard to identify the exact instant that that contamination occurred. The way that we look at false positive cultures in Maryland is we sort our data first by cluster.

Whenever we get new data and then by specimen collection date. And we look for specimen collection dates that are really within a week plus or minus a week of each other.

So in this scenario and this is not the year 20010, it's actually 2010, we find that these specimens were collected at very similar dates and they're in the same cluster.

And that triggers us to look and see where were those organisms collected? Were they collected in the same hospital, were they processed in the same lab?

Because these are clues that one of these two might be false positive. It's not impossible that transmission occurred if one case had been infected for a long time before the specimen was collected.

But it's a high likelihood that something might be happening and this warrants an investigation and if you look at the second cluster in Maryland '10, you'll see that the time the specimen collection was distributed over time.

And that's much more likely to suggest that transmission may have occurred. So are false positive cultures due to which of these methods? The answer is actually all of the above.

Certainly cross contamination in the laboratory is the most common reason for false positive cultures. But bronchoscopes can also become contaminated, be used on one person and become contaminated.

And then that organism is transmitted to another person when the scope is used. That person doesn't come down with active TB but when they're bronch washing is collected and processed and cultured it actually can grow up the organism from the scope.

And we have mislabeling, periodically in Maryland, we had one episode where a patient was in the hospital and on the weekend a doctor who wasn't her regular doctor came in the room and said, "Miss (Jones), I'm very sorry to tell you that your sputum culture was positive for tuberculosis and we're going to have to treat you for tuberculosis".

And in this case we were really lucky because Miss (Jones) wasn't confused or too sleepy to think and she said nobody ever collected a sputum from me.

And it turned out the sputum was collected from the patient in the room next to her. Sometimes there can be mislabeling of a specimen in a laboratory when they process the specimen.

We've had at least three of these and usually they're suspected by a lab or by a hospital, it's very hard to identify from a central perspective.

So causes, we've gone through this, the consequences are fairly serious. The patient gets an incorrect TB diagnosis. They get unnecessary toxic anti-TB treatment and sometimes in our case we've had patients that have been treated for an entire disease, for six months before it's determined that they had a false positive culture.

There are delays in diagnosing what's actually happening with a patient, which may allow that condition to get more severe and although false positive cultures account for somewhere between 1 and 5% of positive cultures, it's also possible to overestimate the TB case rate.

And so these are concerns but the first concerns are actually urgent and we try to jump on anything that we suspect is a false positive culture immediately in our program.

And then finally I just want to review quickly how we can use TB genotyping for program evaluation, we can track recent TB transmission over time by looking at the number of new patients in a large cluster.

Looking at the total number of new clustered patients or the proportions of new clustered patients. And the total number of new clusters.

If we see a new genotype in our program area and two people have that organism and we haven't seen it before it's highly likely the transmission occurred between those two people which we can only know once we investigate that cluster.

So this is - just shows you a large outbreak, we had a transgender outbreak that actually occurred in other states in the east coast as well as Baltimore.

But you can see that the sort of epicenter of that thinking of the earthquake yesterday, the epicenter was 2005 but in 2009, ’10 and ’11 we haven’t seen any cases with that pattern.

And so we know that that outbreak is over. And you can also look at our reduction of the new cluster cases or their proportions of new cluster cases over time and that suggests that recent transmission is decreasing in our state.

It shows that the program is working. And I think that’s the end of my presentation. David I’m going to turn it over to you for questions.

David Ashkin: Wendy, sorry about that, I just turned the mic - that was fantastic, Lauren, outstanding. I mean I really, really appreciated both your presentations and what I’d like to do now is if it’s okay with the audience is I’d like to first go over how to ask questions.

Because I think we’re going to have a lot of active discussions, I know I have a lot of questions I want to ask. But obviously we want you guys to ask.

So there’s a couple different ways to do it, one is that you can email us and if you look on your screen you have a way to ask a question via the chat or the second way is please ask a question which we really appreciate over the phone.

And what I’d like to do now is turn it over to the Global Crossing operator who will explain how to ask a question over the phone.

Operator: Thank you. To register a question via the telephone please press the 1 followed by the 4 on your telephone keypad. You will hear a three toned prompt to acknowledge your request.

If your question has been answered and you would like to withdraw your registration please press 1, 3.

David Ashkin: And then lastly for our audience here at AG Holley if you have a question please just raise your hand and just tell me and we'll get a mic to you.

So Wendy I actually - I'm going to start with you if it's okay. I mean we've really been using genotyping more and more here in Florida and we've had some interesting cases.

And the first thing I'd just like to comment on Wendy which I always get a little upset is you know you guys always bring up the whole idea of how we get cross contamination through bronchoscopy.

I mean you're always blaming it on the pulmonologist that we caused the problem. I just want to ask, that those studies were done by a bunch of infectious disease guys and public health guys up in Maryland if I'm correct.

Wendy Cronin: No, we had transmission through a bronchoscope, that wasn't contamination.

David Ashkin: I'm sorry?

Wendy Cronin: We actually had transmission of TB from a bronchoscope.

David Ashkin: Right, but see you're always blaming pulmonologists, I'm sure there's a lot more transmission, I bet you that bronchoscopy is not the number one mode of transmission of TB, that's just a guess of mine.

Wendy Cronin: I think that might be true.

David Ashkin: Actually you know we've had recently a couple really interesting cases that genotyping has really helped us out. And you know one of the big problems we have clinically all the time is with - I don't want to say cross contamination, but let's say false positives.

And we recently had a very interesting case where we had a patient who is awaiting a heart transplant actually and was having very, very large sore - fusions and had to have the fusions tapped.

And on one of the taps which was AFB negative, it was a trans state, what happened was is that suddenly about two months after the sore fusion was tapped a culture comes back as TB.

And because of this positive culture the transplant team would now no longer transplant this patient because you know obviously with active TB. And you know the patient was sent to us at AG Holley to try to maximize meds and in the course of trying to you know evaluate the patient we realized that the clinical scenario just did not make sense.

This patient was actually a schoolteacher and always had PPDs done every year, her PPDs were always negative, all of her - you know (igras) were always negative.

And we came back and we sure enough did a genotyping and interestingly enough she matched only one other person from the same area in Hillsboro, but there could be no epidemiologic link that we could find in that case other than something that was very interesting, the two patients were at different hospitals.

But interestingly enough the hospitals actually sent the specimens to the same lab. And I guess my question to you is that in the process - then once we - we went back and looked you know both processed at the same time, interestingly enough the other patient was always culture positive.

And the only time that the patient ever had a negative culture was on the day that our patient had the positive culture. So we suspected that that was the day they switched specimens or they entered it differently.

But I guess my question to you is something that we face here at - in Florida is that we try to review our links, you know and what this case taught us is that it's not that simple.

You know we tried to look at the cluster cases and we try to screen them quickly but I think you alluded to that sometimes these cases are very hard to pick up as a true cross contamination or false positive.

And that even using labs, because we used to just look at where they were submitted from and if the two hospitals were the same we started to look closer.

But in this case what's making it more complex now days is more and more hospitals are not processing the specimens and sending it to a similar lab.

But the results come back to the hospital. And I was wondering if you have any suggestions on when you were looking at before ways to - you know what you look for to you know suggest clustering, do you look at where the specimen came from or more importantly where the - which lab processed it?

Wendy Cronin: We do both. We look to see where it was collected. We collect information on the originating lab for this - for the specimen actually because sometimes what will happen is the specimen will be collected and maybe they do a direct smear at a hospital and then they send the specimen to the state lab for culture.

And the contamination actually occurred at the hospital where they did the smear and not at the state lab although certainly we have rare episodes of contamination in the state lab.

I mean some of the clues of false positives are that they take a long time to grow up like the example that you discussed where it was eight weeks almost before you finally got the positive.

Because usually there are very few organisms in the culture. So we go through both - to answer your question directly we go through both. We like to look at the lab where it was processed and the place where it was collected and we also - if we suspect that a false positive could have occurred, we try to talk with the provider to understand what the clinical picture is with that patient.

Because we want that person to think about whether this could actually be TB or not and sometimes you know with HIV patients, that's not an easy thing to determine and so you really need to be very careful about your investigation.

And we've also had false positive cultures in patients who do have TB but just that particular organism was not their organism. And we can test that if they had a prior organism or sometimes they become diagnosed as clinical TB because the provider just feels that it's - their symptoms are similar enough and they're afraid to stop treatment.

David Ashkin: I think you bring up a very interesting point, you know and we do the same thing which is when we start to suspect the possibility of a cross contamination or a false positive, whatever you would like to use and each situation is a little different.

We usually look at if the person was consistently smear negative because I think you'd agree that normally if the person is smear positive it's less likely to be a cross contamination except in cases like this where you may have mislabeled it or you may have switched the specimen.

And then just like you said that the clinical scenario just doesn't match. But one interesting part we found in this case also that kind of complicated things was that the clinicians who originally took care of the patient back in the hospital were not the clinicians who later on made the diagnosis.

And that's another issue we're having is that there's a lack of - there's not the continuity of care that we would like and I guess my comment on this whole thing is that often I think it's an important message for all of us in public health, we're the ones, it's the TB clinics and the clinicians in the clinics that are going to have to go back and make that diagnosis because a lot of times the care is somewhat fragmented.

And I think that's why genotyping is so important and so that you're bringing out and I think both you and Lauren brought out very importantly is it's only one piece of the puzzle. I think we need the whole other end of going back clinically, making sure it's appropriate.

So thanks, we have a question that came in from our email and says, and I'll turn this over, if it's okay Lauren, I'm going to let you answer this. It says can we reveal names identification information during cluster investigations?

And again Lauren or Wendy which ever, whoever wants to answer it please go ahead.

Lauren Cowan: Actually Wendy I think you'd probably be more appropriate for that.

Wendy Cronin: Okay. That's a good question. We certainly in Maryland are not allowed to do that and I think because of confidentiality you really, really have to avoid giving a name up front.

You try to get the patient to name another patient and we also find that we don't name the settings for example if there's a school where you believe transmission occurred between those two patients, you don't name the school.

You try to get them to give you the name of a place to confirm that that person might have been in that school or that store.

We had a store or a church, sometimes if we're pretty sure there's a link and we just can't get it, we might you know give a list of names of workplaces and the name where we think it might have occurred will in that list because we know somebody was in a - oh I don't know, maybe they were a security guard or something.

And they work somewhere and we just want to throw out the name of a bunch of security agencies to see whether we could get them working in the same place.

But they can't tell from that list which one we're really looking for. I know that in some parts of the country, I think New York City and I know Alabama

has used this in the past, people have shown photo sheets of people including the person that they're interested in finding out if there's a link.

And it may have 25 people on the picture, on the set of photographs and one person is a real TB case and the rest are all people who work at the health department or other people.

And they try to just say do you know any of the people on this picture, have you spent time with any of the people on this picture. But we also are not allowed to do that in Maryland.

So you really need to be careful about confidentiality and you have to work within your state confidentiality laws to make sure that whatever you're doing is within the state law.

David Ashkin: Wendy can I add - can I ask you something about that just to go along with that? Again we recently had a case where we had a few patient who had a multi- an excessively drug resistant form of tuberculosis and one - when we went back to look at the genotyping the patients matched, but had no epidemiologic links that we could find whatsoever.

One was a young individual who had absolutely no links whatsoever and it was a purely - it was truly a case of MDR TB but none of the classic you know risk factors, never had TB before, never traveled outside the United States.

And they matched another patient who interestingly enough was also at the time a patient at AG Holley, or not at the time but about a year before and actually went back to both patients because we had no idea and we asked if it would be okay for them to meet.

And both of them agreed that it was okay and that they waived their "confidentiality" and we actually had them meet. I was wondering how you would comment on that and naturally you can yell and scream at us about that.

But they both felt that it was important to try to figure out where the transmission came about and we also thought it was important because there may have been other individuals in that location that may have been exposed.

A comment by you?

Wendy Cronin: Well I think that's a really interesting approach, we have never even thought about doing that. But for MDR you know if we ended up with an MDR cluster like that we might because that's a huge concern.

David Ashkin: Exactly, this was a young kid who had absolute no risk, here's the bottom line, you want to hear it? We had them meet, they both were very agreeable, they sat and spoke for about an hour, an hour and a half.

And still at the end of it we could not figure out what the link was.

Wendy Cronin: I'd be very curious to know whether they were foreign born.

David Ashkin: Well actually the first individual was, the second was not. It was a patient who was - the young gentleman was a US born no risk factor which I was going to bring up the second point, question if I may and for either Lauren or Wendy, is do you ever - you know one of the things that we found very helpful is she used the genotyping to try to track the geographic location of the strain.

Interestingly enough the strain that the young gentleman had was one that was very common in India which is where the first patient was from.

So again it didn't speak for a US transmission, I was wondering how - do you guys ever use the genotyping to try to figure out you know maybe potentially countries of origin?

Wendy Cronin: Lauren that one's yours.

Lauren Cowan: Yes, and so the TB complex can be divided up of course into the animal strains and then there's four lineages of TB (sensistricto) and those are the indoceanic strains which are prevalent along the rim of the Indian Ocean.

The east African Indian strains which are prevalent in east Africa and northern India and then east Asian or commonly known as Beijing which is very prevalent in southeast Asia and then up into eastern Asia and then finally the Euro-American which is of course very prevalent in the Americas but also in Europe.

And so we can assign our genotypes to these different lineages and that does give us information. It never proves anything but it does like you say suggest that if a person has you know a strain that we would call Indoceanic Manila and is from another country such as somewhere in South America, you would have more of a heightened interest in questioning where that strain came from in that foreign born person.

There was an MDR outbreak that I just love in that California identified two cases and it was a strain that was prevalent in India again but neither of these two patients were from India, they were both foreign born but not from India.

And they did end up finding a source case outside of the state that was from India so yes, that information can be used very effectively and it is somewhat provided in TB (gems) with the genotyping data.

We do indicate the lineage and then a sub lineage such as Vietnam, India, Manila, to give you kind of an indication of where that genotype is prevalent.

David Ashkin: Lauren, first of all I love your statement that you love that MDR case, I love that you two are TB nerds like the rest of us. That - just out of interest was that case outside California, was that from Florida because we still are trying to figure out where that link is. I'm joking.

Lauren...

Wendy Cronin: I think you should check Georgia.

David Ashkin: Oh really?

Wendy Cronin: No, I'm just kidding, that's where Lauren is.

David Ashkin: Actually Lauren another question for you and just a - not a basic question but an interesting question, what's the most common strain in the US?

Lauren Cowan: I saw that question so I quickly opened up my database to see what it might be and it definitely is - so hoping you'd give me enough time to do it. The most prevalent ones are members of the Beijing family.

And some of the strains would be strain 210 if anybody's seen that one in the literature, that one has been circulating in the United States since the mid-90s when we first started doing genotyping.

There are some strains that are very common in foreign born patients, so we believe that they are circulating and there's some genotypes I guess I should say that are very common in diverse foreign born patients that we think are circulating overseas and we are just not differentiating those genotypes into different strains.

And then the southeast the most common genotypes are Euro American (acts) which is the two band family and so some of you know about the two band IS6110 powder and that we couldn't do anything with for years.

Those are still very prevalent strains in the southeast.

David Ashkin: Thank you very much Lauren. Just a quick before we go to the next question but I have on my screen here, I have a couple people raising their hands on the computer which I really appreciate.

If you guys would - we would appreciate it if you guys want to ask a question live just tell the operator on Global Crossing and you know and we'll what do you call it - and this way we can hear your voices which is always much better.

So can I ask the Global Crossings operator one more time to repeat how to ask a question live.

Operator: Certainly. Ladies and gentlemen to register a question over the phone please press the 1 followed by the 4 on your telephone keypad.

David Ashkin: Thank you so much. We have a question in the AG Holley audience, Dr. Jesus Ortiz, you have a question?

Jesus Ortiz: Yes, can you hear me? Good morning. As I'm listening to this lecture I simulated some ideas. You know when we talk about tuberculosis, usually you're talking about a particular strain with a particular patient, a particular genotype.

But I'm just thinking in a scenario for example in a multi-cultural area where people would be crowded and exposed and let's say an immuno-compromised individual, if you have more than one strain exposure to that person, could they actually have two or you know theoretically more than two strains of tuberculosis at the same - in that same individual affected?

David Ashkin: Wendy or Lauren you want to answer that?

Wendy Cronin: I can take that and Lauren you can add to it. The answer is certainly in countries where there's a lot of TB circulating they have found multiple strains in a person.

And in this country even when they have cultured different sites from the same person occasionally they have found a different strain.

We still generally assume that the one we get first is the one they have and so we don't work from the assumption that that person has multiple strains.

But it's definitely possible and I think the higher the likelihood of exposure, the higher possibility that you might have more than one strain in the same person.

Lauren Cowan: And we can get hints of those multiple strains from the genotype, we end up with a pattern that we just don't expect and that is usually indicated in your genotype comments that this is a mix or possibly a mixed strain.

And then if people want to dig deeper there are opportunities perhaps to do that.

David Ashkin: Thank you very much, I see here that I have a question from my boss, from (Donna) so my job is on the line right now so if I don't ask this I'm in trouble.

But she's saying that she's very intrigued by the statement that you're working on integrating questions about social settings into contact investigation use.

And obviously the field will be very interested in seeing what is being developed and she's curious about the length of time required by the lab to report out genotype results.

And is it realistic to assume that when they will be able to integrate results into ongoing CI, and actually there's a couple different questions that relate to the fees and how long it takes to do the spoligo and the MIRU and wonder if Lauren maybe if you would comment on that.

Lauren Cowan: You know I don't think it's so much the speed of doing the spoligo and the MIRU, it's just that right now we genotype off of cultures. And so the routine is for labs to identify in isolets a culture positive isolet that they need to perform drug testing on.

And then they usually set up the midget drug testing procedures and then send the culture positive control from the midget drug testing off to the genotyping lab so that they're always having a culture in their lab.

So it's - that's really where your time comes in because I would imagine that would be at a minimum four weeks. And right now we just - there's so much to chaotic DNA, in sputum that we really don't have a way to perform genotyping on those.

Wendy Cronin: And I'd like to add, we feel the same way that the turnaround time for us has been pretty consistently two weeks and sometimes less. But it does take a while to get those isolets out to the lab.

And we tend to batch them because of just short staff numbers, we try to move them fairly quickly but there are delays due to that as well.

But in terms of the actual contact investigation there's always the issue of how long it takes for the secondary case to break down.

So if you have a situation where I described the first case as being infectious for a long time, and the secondary case as actually coming down with active disease before the first case is diagnosed, in that case the genotyping and the time of the cases might occur around the same time as the contact investigation.

But for example in the case of the bronchoscopic ones which David mentioned so the first case was bronchoscope to be diagnosed and then the second case was bronchoscoped and it was seven months before she finally came down with active disease.

People who get infected don't come down with active disease usually before three months. And so you've kind of got two things going on, the contact investigation wants to pick up the people with latent TB infection to prevent them ever getting disease.

If you do have a contact who has active disease then the genotyping might show that yes they do have the same pattern. But I see genotyping as helping contact investigations more by saying,wow, this contact investigation needs to expand.

And I can give you an example of that, we had - it was very unusual, we had transmission in a store very small, very crowded store and the cashier was highly infectious.

And two patrons of that store came down with the same pattern. Now we would never have conducted a contact investigation in a store. We did conduct it among this cashier's coworkers but we never would have thought to do it among the patrons.

And when we got the genotyping results this is one of those cases where you put a bunch of places on a list and then you have - see if they can pick out this one store where the cashier was.

We found out that those two patrons had been in that store, one of them had been there quite frequently.

One had been there fairly rarely and so we went back to the store and we actually got a list of other customers and tracked them down and did a full contact investigation on store patrons.

So I think that's maybe a more active way that genotyping can help contact investigations although it also can tell you that yes, these two people that you identified as contacts do have the same pattern.

I don't know if that's helpful.

David Ashkin: Thank you. You know we're being - we're getting - you know first of all you know there's a lot of - you know first of all I'm getting a lot of comments as you are that they really appreciate the practical implications and I think one of the big concerns I'm seeing in the questions is the length of time to get back to where we were before.

You know but in my experience, our experience here, it's - and I would like to get Lauren and Wendy's comments.

But we've had experiences where we needed a result quicker and we were able to call the labs and they really have been able to help us out with essentially getting us results quicker and I think it's always important to know with communication between the labs.

And I was just wondering you know again I think a lot of the strains coming back within the normal period of time within two to four weeks I think is very acceptable.

But in certain cases where we needed a quicker result, I mean the labs have been able to really expedite it or at least try their best to expedite it.

Lauren do you agree with that or Wendy do you agree with that?

Lauren Cowan: Yes, I'm thrilled that you said that and the labs are always willing as long as they are able to triage different things for important reasons and one of the most important ones is for false positive investigations where you really need a result quickly.

And they can also provide interim reports so spoligotyping is much faster than MIRU typing, our throughput is much quicker. So we often have that result first but hold it until we have the complete MIRU VNTR results.

And so in a false positive investigation a spoligotype may be all you need to make the conclusion that you need to make and then you'll get the additional information as it's available.

So interacting with the labs as much as they are able is a wonderful thing to do and if you ever have a question about a result, they can you know look at the raw data and look for things that perhaps aren't immediately apparent.

David Ashkin: I totally agree and two things that you know just to comment on just to follow up, what is usually with the lab contaminations or cross contaminations, you know or false positive, I'm very capital how I state these because the lab gets very upset when we call them lab contaminations when they're really not the lab's fault, you know?

But the bottom line comes down to is usually when we're dealing with a false positive it's a little easier because there we're working off a culture. You know usually we're dealing with two cultures that were positive.

But to comment back on what you said and I totally agree and I think we need to emphasize this is that the spoligo results come out faster. But like you said they're usually held.

But in cases where we need the results faster we can request the spoligo with obviously the disclaimer that the results we're about to do may not be complete and we may not, the MIRU's may be different.

But at least it gives the program, at least the initial impression of how to proceed or where to proceed. Do you agree with that Lauren?

Lauren Cowan: Definitely.

David Ashkin: Yes. You know there - we have a question here that I think I understand but it's says I've heard that there's an initial genotype into families first and only fingerprinting done if it is requested by the state.

Is this true and I'm not 100%, I think what they're getting at - well actually Lauren, I'm going to let you try to - do you have a comment on that, or first of all I guess...

Lauren Cowan: I can go ahead and elaborate a little, so yes, we do spoligotyping and MIRU typing and present a very good picture of the genotype of that strain. Especially when we were only doing 12 loci the genotype wasn't always discriminatory enough.

We weren't always breaking it up into the different strains. There were some very, very large clusters, some of you may know the strain PCR or the genotype PRC type 2. And so IS6110 was very effective in adding a different additional information to that cluster.

Now that we have the 24 loci the role that IS6110 plays seems to be diminishing. Very few people actually request it. And we do use the

genotypes to assign to the lineages or the families that I mentioned above so that would be kind of the top of the hierarchy, then you would have the spoligo and MIRU.

And sometimes you may or may not have a need to add IS6110 to the picture. As far as the number of requests for IS6110 nationally I think it's less than 250 a year, so it - the - it's place is definitely decreasing.

David Ashkin: And again if I'm correct Lauren, most of those requests I believe for the IS6110 is when we're trying to compare it to all the strains where IS6110 was done only, is that correct?

Lauren Cowan: That happens sometimes, yes.

David Ashkin: Okay, thank you. And we have another question here and I think it's more related to Florida so I can at least begin to answer it and then please make comments.

But the question is, is genotyping being performed for all cases, all TB cases in Florida and then you know they ask about how long does it take to obtain the genotyping results.

Just a quick comment on what we're doing here in Florida, we actually have assigned one person in our lab, Richard Doggett who actually assures that every positive culture that we have in our Florida state lab as well as trying to acquire the cultures from the community and making sure we have at least one culture that's representative of every case.

All those then Rich's job is to make sure that we send all those off to our regional genotyping lab and then most importantly that the results come back and then get entered into our system.

So the question is, is it being done on all cases in Florida? We're trying. I have to say to you obviously a couple of cases, the cases that are obviously culture negative or clinical cases.

Obviously there's no genotyping done and I'd like to say that we're able to get every culture for every case but that's not always true, sometimes the cultures are not viable, we have other issues.

But we're trying and maybe Wendy or Lauren you want to comment on how other states are handling or what I just said, kind of similar to what's going on in other states or other areas?

Wendy Cronin: Lauren I don't know if you have the answer to this but I know nationally when I used to talk about genotyping that nationally about 60% of national isolets on culture positive patients were being received and genotyped by the regional labs.

And now it's up to over 80% and Florida was one of the lowest, Florida was something like 22% and then every year Florida has increased rapidly and they're one of the high contributors now.

So I do know that about Florida, Maryland has hit 100% for the last two years and we've been always over 95% since we started in 1996. But it does take work.

It's easy to get isolets from the state labs but if your state lab doesn't have all the isolets it takes a lot of work to get them from the commercial labs and the hospital labs.

It just takes forming good relationships with those laboratories, communicating with them, identifying a contact person and getting those people to send the isolets in.

And we also have in Maryland a state code that says that one example of all culture positive patients has to be submitted to our state lab and our commercial labs are very quick about that now.

That changed things a lot for us. It meant that they automatically submit to us anybody that has an address in Maryland. And that allowed us to collect those isolets better.

And then our state lab submits all the isolets to the genotyping lab.

David Ashkin: And you know Wendy I really want to thank you for pointing out Florida's poor performance we really do thank you publicly for that.

Wendy Cronin: I was trying to point out your incredibly improved performance, you're one of the high performers in the country.

David Ashkin: Well actually two things just in defense.

Wendy Cronin: It's a lot of work.

David Ashkin: Well in defense first of all when we were doing our own genotyping we may not have let's say been sharing that which was a mistake and we tried to correct that.

And now we're part of the regional just for our Florida participants here. But the second thing is that we all believe that by putting one person in charge which is Rich Doggett, if we continue to do poorly we can just fire him which was a much easier way to handle the whole situation.

So that was our solution to that.

Wendy Cronin: Let me ask you, is he a laboratory end or is he a program person?

David Ashkin: He was actually an epidemi - and again I want to make sure that I got this right, I may mess it up, hopefully Rich is not listening.

But he was with our TB program and more on the clinical side, on the epidemiology and now his position in the lab as a liaison between the lab and our TB program to make sure that we're getting you know every - you know best as we can to make sure we're getting as much genotyping data as possible.

Wendy Cronin: That's great.

Lauren Cowan: There was one more caveat to the whole thing, there were some microbiology issues not quite getting the amount of sample to Michigan that needed to be done.

And so Maria in Florida has worked with the Michigan lab to improve the capability of the isolets and so even though it looked like they weren't submitting it, it really turned out that there were lots of isolets with no results.

David: I appreciate - Maria has done a great job and I appreciate that very much Lauren, and you're right, we had some other issues that were trying to - you know obviously we all want - I think you know one of the other things I think we should talk about is, I think all of us in TB control want to develop this national database.

And like you've pointed out the more specimens, the more complete the database, the better off it is for all of us. And that was definitely our impression and that was definitely our goal.

And that was why we moved and I want you know thank the Bureau of TB, (Jim Cobin) and Mack for supporting that and making sure it got done.

We have a question here from somebody who has a very interesting situation and they're asking what do one of you think maybe the explanation for a family of three sisters and one niece, all diagnosed and on treatment at the same time with three different genotypes, only two sisters had the same genotype.

What would be your comment on that?

((crosstalk))

David Ashkin: It make me nervous here, I just lost my morning coffee just now. You guys can't say hmm.

Lauren Cowan: You know it's always hard to comment on three different genotype results without seeing those genotype results. A lot of times people will say something with a dash in it is different when it's really not.

And so without actually looking at the data and knowing the background, it's very hard to make any type of comment on that. But we are always you know here at CDC we're always interested to see that data and to discuss it with people.

TBgenotyping@cdc.gov.

David Ashkin: Go ahead Wendy.

Wendy Cronin: I can add a little bit to that, so Lauren's point of looking to see whether one spacer in the spoligotype or one locus in the MIRU patterns is different, could mean you're really working with the same bug and it's just hit one of its mutation periods at the time of transmission.

So that could be one reason. But (Chris Bradon) many years ago using RFLP was able to show that in families of foreign born folks two people could come down with the same - two people could come down with TB around the same time and the patterns would be different 25 to 35% of the time in foreign born families.

And there's some evidence which I don't want to get too far along with this but I know (Phil Hopal) has also mentioned it, there's an enzyme in TB that seems to stimulate a latent organism to active.

So if one person has active TB it appears there is something that is sent out that stimulates the other organism to active in another person and I know there's some research into this trying to understand what's happening.

And so it's possible that there's actually an explanation for several people in the same household having different organisms. We've seen it in Maryland we're conducting one of the TB epi studies task orders and we'll be analyzing that data shortly.

And one of the things we're looking at is people who are epi linked, have a close epi link but a different genotype pattern just to see where it happens and who it's happening with.

And to understand how different that genotyping pattern is from the main cluster pattern.

David Ashkin: I also think something else, it also may tell you that this is a family that's obviously not very close and don't eat together or hang out and they're just very unlucky when it comes to TB.

So maybe we're learning more about our genotyping and how - in dysfunctional families or functional families, you know?

But I'm not so sure, but to kind of further what you were saying in a second ago, though we also use genotyping a lot as you kind of alluded to with reinfection versus reactivation but from a clinical standpoint I think that has a lot of clinical implications.

I think you agree that if you have somebody who just got reinfected it tells you a lot more about person's immunity and you go clinically through a

different algorithm that is if this is a reactivation, it helps us clinically tell like A, you know did the person obliviously to review did the person really take their DOT B, did they have issues with their drug levels or C you know did they develop resistance?

So you know we also - you know we kind of alluded and not quite in this case but that whole issue of reinfection versus reactivation, I think clinically also has a very important role in how we're going to handle these cases.

And I think again we always try to review that, you know? We have another comment here and I just you know it says that any recommendations on two patients or any ideas or you know what's your take on two patients who are co infected, who were diagnosed five months apart with the same spoligotype, so Lauren, Wendy do you want to comment on that scenario, what you think maybe - what you think may have gone on?

Lauren Cowan: Wendy?

Wendy Cronin: Can you say the first part of that again, they were diagnosed five months apart?

David Ashkin: I apologize about that, it's two it sounds like co infected individuals, I take it two individuals who are probably infected with HIV and TB who were diagnosed with TB five months apart and they both have the same spoligotype.

You know from your point of view, you know what do you think probably went on?

Wendy Cronin: That's where you get into that you need the shoe leather epidemiology. First of all I'd like to see more than the spoligotype, I'd like to see either the MIRU24 or an RFLP because spoligotype by itself is not a very good differentiator of clusters.

And then the next thing would be the know, you know the field epi part would be were they in the same clinic, the same HIV clinic at the same time or were they around each other at the same time?

Because as we know TB if one person is infectious and a second person is exposed, five months could be a rational time that they would break down with active disease after exposure.

So I think the scenario could imply transmission but it would be helpful to have more genotyping information because spoligotyping is very sensitive and not specific and also it would be helpful to know whether they actually spend time together.

You - so this gets to the point of really being able to do a more in depth investigation to actually understand things. You can guesstimate but you can't actually say what happened without more information.

That's the fun part. I mean it's not just fun, it's useful to programs but it is detective work and it's kind of interesting to get into these situations and understand what really did happen so that for example if you had transmission and HIV clinic what infection control practices are they carrying out and do you need to intervene?

David Ashkin: And I think you'd agree and I mean this question actually - and this comment actually goes to another question that we've been seeing here in Florida, I

wonder what your take has been, when we talk about not just HIV but immuno suppressed individuals we're seeing more and more issues with our transplant patients.

As we're seeing more centers in Florida do transplants we're getting more cases of TB and genotyping becomes very, very important because the question always comes down to was this a reactivation in a patient that was already infected with TB prior to the transplant?

Or was this somebody who actually caught TB from the transplanted organs and we've had a number of cases now where we're seeing TB being transmitted through the actually transplanted tissue itself.

So I was wondering if Wendy or Lauren wanted to comment on that.

Lauren Cowan: I know CDC has been involved in some investigations of that same type and yes, it does occur and I believe that there's a review of that either coming out or is already out to encourage doctor's managing the treatment of these patients to use genotyping information to use country of origin information, to use genotype lineage information to try and figure out the puzzle.

David Ashkin: And I agree and actually that question in some ways was a real - I have to admit it was kind of a teaser that you are right. That there is some information coming out and we're going to - one of our future grand rounds is actually going to be about TB and transplants and the role of genotyping that you guys have done a great job of really I think making it very, very complex subject very understandable and very practical.

And with that what I'd really like to do is I'd like to thank Lauren, Wendy, thank you so, so much for what I think was an outstanding presentation and thank you very much for sharing your expertise.

We really appreciated it. We've been getting a couple questions about the slides, will they be available. As you guys know this Grand Rounds will be archived onto our site so that you can listen to it and enjoy it again hopefully in the future or if one of your colleagues missed it.

Please go to our website and please see the archives. As far as the slides I believe again if you go on to our website or send a question or a request to our web master they will provide you with the slides.

Other than that I really want to thank everybody for joining us today. I want to remind all of you please go back and fill out your CMEs and CEUs so you get credit for this great program.

And I want to remind you about some things that are coming up. For all you guys from September 12 to September 15 we're having our comprehensive TB course here at AG Holley. We'd love you to join us, hopefully there will be no hurricanes during that time.

If there is a hurricane if any of you want to experience it first hand please come and join us. On September 16 we'll be having the TST train the trainer, on September 29 through 30 we have our TB program management course in Kentucky and we're looking forward to seeing all you guys in Kentucky.

Our next grand round is going to be October 12 and it's actually our national grand round so it will be broadcast and we're going to have - we have the distinct pleasure of having Elsa Villarino from the CDC join us and with some

very exciting news and I think we'll be very, very relevant to all that's in TB control which is the discussion of the three months of once weekly rifapentine in INH for the treatment of LTBI.

So that's going to be really, really exciting. And then on October 18 as part of the Southeast National TB Control Meeting in Charlottesville Virginia as long as they don't have an earthquake there will be doing LTBI revisited, new diagnostics and treatment guidelines.

So we really appreciate seeing you soon, we appreciate you joining us and other than that please stay safe and for all you guys who are interested at 12:30 we'll be doing our morbidity and mortality and for you who have already registered please just click on the link.

And we'll be talking to you guys and for the rest of you we hope to see you real soon. So from all of us at the SNTC, thank you Wendy, thank you Lauren for a great presentation and other than that we'll see you guys real soon, thank you very much for joining us.

Wendy Cronin: Thank you.

END