FTS-CDC-EPO

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12:00 p.m. CST

Coordinator — Good morning and good afternoon, and thank you all for holding. I'd like to remind all parties that the conference call is being recorded today. If you have any objections, you may disconnect. All parties are on a listen-only mode until the question and answer session. I would now like to turn the call over to Miss Sophia Glinos. Thank you. You may begin.

S. Glinos — Thank you. Good day, everyone. Welcome to our teleconference What's New in Tuberculosis? This is Sophia Glinos. I'm the State Training Coordinator at the Wadsworth Center New York State Department of Health in Albany, New York.

A few program notes before we begin. CDC, our planners and our presenters wish to disclose they have no financial interests or other relationships with the manufacturers of commercial products, suppliers of commercial services, or commercial supporters. Presentations will not

include any discussion of the unlabeled use of the product or products under investigational use, with the exception of Dr. Max Salfinger's discussion on drug testing and fingerprinting of end tuberculosis. He will be discussing the methods for molecular drug arrays and fingerprinting.

After the program, each participant needs to register and complete an evaluation form. Documenting your participation helps us to continue to bring high-quality training programs in a variety of formats. To do this, go to www.cdc.gov/phtnon_. The password is TB. Again, the Internet address is www.cdc.gov/phtnon_, and the password is TB.

When you've completed the registration and evaluation form, you'll be able to print your CEU certificate. You have until January 14th to complete this process. These instructions are in your original confirmation letter and the general handout. They were also e-mailed to each site rep this morning.

If time permits, the end of the program will be open for questions. You are on a listen-only line. We cannot hear you. You can only hear us. If you experience any problems with the line during the conference, please

press star zero. This will signal the attendant that you're having a problem.

Again, welcome, and thank you for joining us. We have over 65 sites from across the United States listening to the teleconference. Today's speaker is Dr. Max Salfinger.

Since 1992, Dr. Salfinger has been with the Wadsworth Center Clinical Mycobacteriology Laboratory, has been the laboratory director. Furthermore, he is a Professor in the Department of Biomedical Sciences at the SUNY School of Public Health in Albany, and is an adjunct Professor of Medicine at the Albany Medical College. He is a member of the WHO DOTS+ working group. It is my pleasure to introduce to you and to welcome our speaker, Dr. Max Salfinger.

Dr. Salfinger Hello, everybody. My name is Max Salfinger, and the topic today is What's New In TB? And the question already comes up, what does new mean? It can mean new in research or new in practice, and today, I would like to focus on new in practice. After a short introduction, I will talk about patient management, drug resistance, TB complex, Healthy People 2010, errors and interferon-gamma assay.

What you can see here is the David Wadsworth Building, one of several buildings, which belong to the Wadsworth Center, which is the New York State Public Health Laboratory. And underneath you can see our Mission Statement: Science in the pursuit of health, meaning as a regulatory agency, we need to make sure that we have always the science on our side.

Tuberculosis is really a special disease and, already, Sir William Osler from Baltimore at Johns Hopkins coined the term "Tuberculosis is a social disease with a medical aspect." That tells us, if we do only the medical aspect, we will never win the war against tuberculosis.

The transmission occurs usually through small droplets nuclei through the air. But we should not forget that there is also another route, the so-called gastro intestinal route, through contaminated milk or dairy products from cattle herds, which suffering of tuberculosis. And in these circumstances, actually, there is less pulmonary disease, rather than extrapulmonary disease.

You have to differentiate between the healthy people, infected persons, and then the really sick people. And the natural history of TB, you need to realize that 10% of people who had at once an exposure to a TB patient,

has a 10% chance to get sick with tuberculosis at some point of life. When the patient has HIV positive status, which is a very strong risk factor for development of TB, then the risk is even higher, 7% to 10% each year. So not any longer for the entire life span, and ultimately, if we don't treat the TB patient, 50% of them will die.

Some data from WHO: about one-third of the world's population is infected. That means that Tuberculin skin tests will be part of this, but there are no symptoms, no disease. About eight million new cases each year occur, and two million deaths each year all caused by tuberculosis.

In our country, United States, we measure, usually, what something costs. And for us here with the drop susceptible TB case, to cure a TB patient with $92, it's just about $22,000. And in the event we have to deal with a multi-drug resistant case, then it goes up to $180,000.

Early '90s, when TB epidemic came back in the United States and we had increased numbers of tuberculosis cases, several public health laboratories developed Fast Track programs. And the bigger ones like Florida, Texas, California, and us in New York, we all have priorities for highly infectious TB patient or suspect of, to make sure that we have the shortest

turnaround time to separate these patient and to finally diagnose and confirm the suspicion. But these Fast Track programs, they vary from state to state, but the overall aim is to really have state-of-the-art laboratory procedures in place and the shortest turnaround time.

When a patient sees a doctor, there are three different questions, which really need to be answered by the laboratory. After history, physical exam and chest x-ray, the first question is, is it TB, yes/no? Second question: …and resistance – yes/no? And new, the time to negativity, what means that the TB control needs to document not only any longer if the patient takes the medicine, but also how long it takes for the culture to become negative. And this is part of the guidelines written by the American Thoracic Society, CDC, Infectious Disease Society, in 2003.

This brings up the issue about follow-up specimen. In the past, we dealt, more or less, only with the diagnostic part and now we are also more involved in the management part. And the follow-up specimen in these guidelines, as I mentioned published 2003, say that we need to have follow-up specimen until two consecutive specimens or culture are negative in the event of…smear negativity, initially at least once a month when the initial smear was positive bi-weekly. And, although in the

guidelines it's not specified, for us in the New York State, we treat every event in a way that we actually collect two sputum specimens, not only one, because we don't know if all the sputum specimens actually are of good quality. And in the event it gets contaminated, then we still have the other one, which can count towards the time to negativity.

The real reason why we need to document the time to negativity is in the event we have initial cavitations on the x-ray and the culture is still positive at month two, that means the patient cannot be treated any longer with a six month regimen. It has to be extended in the second phase from four to seven months, meaning that ultimately this patient needs to be treated nine months.

Now we are shifting gears a little bit and talking about the global village, a term, which was coined by Marshal McGluhen, a Canadian, in the 60s when the TB became more popular, and the true runner at that time was the media is the message. But it also meant that we are more connected, not only through TV, but all the other higher technology we have now available. That means we cannot isolate our TB control programs and not look at what's going on abroad.

One obvious thing is that the foreign born person with TB in this country actually have a higher percentage. In the slide you see the graph from 1994 where only a few states have more than 50% of their TB patients seeing foreign born, and in 2004 there are many more states being purple. In absolute numbers, one would say, yes, the foreign born in the purple bars are more or less the same. And in the blue bars, the U.S. born TB patient actually reduced. But this also tells us that what Osler said about TB is a social disease, is a medical aspect that rings even today, true. Because these foreign born TB patients often times do not have access to healthcare and, therefore, they are for us below the radar screen.

The other issue is the rate is quite different according to the latest data published by CDC 2004 data, the U.S. born rate per 100,000 was only 2.6%, while the foreign born TB patient rate was 22.8%, so almost ten times more prevalent among the foreign born compared to the U.S. born population.

Next slide we are talking about drug resistance. And there with drug resistance similar issues; U.S. born versus foreign born in terms of INH. U.S. born is 4.5% via drug resistance, and foreign born is at 10.4%. And in the MDR, although it's lower, it's still a factor about times two; U.S.

born patient 0.6%, and foreign born at 0.3% of MDR; or for the entire U.S. TB population, INH resistance in 7.8% and MDR in 1%.

The gray area-- I have to go back to this prevalence of any drug resistance among new TB cases, the latest data published by WHO, and you can see the darker the color, the higher the percentage of drug resistance. But what is stunning to us that the most part of the globe in Africa and in Asia, there are gray colors, meaning we don't have any data about drug resistance prevalence.

And another slide borrowed from WHO, in their third report about drug resistance, they claim hot spots in Eastern Europe, Central and Southeast Asia. And since we get more and more immigrants into this country, we need to be aware that these patients are not bringing only labor and whatever, they also harbor TB…

The treatment of MDR TB requires a regimen of three to four drugs to which the isolate is susceptible, and it's not any longer only six months treatment or nine months treatment. Now we are talking about one and a-half or even two years beyond culture conversion. And this is one reason why in New York State, we have the requirement that drug resistant needs

to be confirmed either by a second method or a second laboratory because we want to make sure that this laboratory result is actually correct.

Interesting data published by Floridians in just 2001 about their cure rate of MDR TB patient and it really depends who takes care of these patients. If physicians in the community took care of the patient, then the cure rate was only about 50%. However, when the expert in the TB hospital at AG Holley in Lantana took care of these patients, it increased to 80%.

And the drug resistant issues tell us that we really need to be faster in terms of turnaround time. But how fast is fast? In this resistance diagram you see in the bottom our TB analysis, which requires a PCR reaction, and then a DNA sequencing in some of the applications, is currently the fastest method; a few days turnaround time, that's it.

The radiometric, the BACTEC 460 and any of the non-radiometric processes, it takes about one week after a culture is positive. The arger base method there three weeks after the culture is positive, and that would contribute on Lowenstein-Jensen, which would be four weeks, or we can simply wait until the patient is not doing better. Then the clinician also

knows, either the patient didn't take the meds or the organism is drug resistant.

If the laboratory is still using arger-based method only, then in these times this is really too slow and is also mentioned as such in the guidelines issued by the CLSI form and NCCLS issued in April of 2003, that primarily we should do the growth-based system. But there is another catch with these guidelines because they mention that one should test initially, INH, rifampin, and Ethambutol only, and if rifampin or any other two drugs are resistant, then one should go on and do more compounds. And they specifically mention that the state public health laboratory is the reference laboratory. So if we go then and add the arger-based method, then we slow further it down. And for that reason, I think we need to have a more rapid diagnostics in the laboratory.

This is just a figure about the RPB analysis, and we check against the vile type and the code on 526, which encodes the histod in the vile-type strain, meaning susceptible strain, was replaced by a GAC, which then in turn encodes aspetate, which is a change now in the protein and it is a resistant strain.

This is a list from a…article written by Jim Musser and colleagues, already seven, or even eight years ago, and you recognize that the code on slide 3-3, I placed a large arrow with a giant explanation mark. And this particular …will not show up in the backpeck or others processed at medium as resistant because the MIC is too low. And for that reason in our laboratory, we screen in the backpeck 0.5 in addition to the 2.0 concentration to catch this rare mutation. And in the past, we have seen maybe three to five additional cases, and we even used this particular mutation in the proficiency testing, which then demonstrates that the regular assays, the non-molecular assays, do not catch this strain as resistant.

This is an overview about the molecular drug resistance at testing in terms of the correlation of the gene with the mutation with the gold standard with the arger portion and you can easily see revamping. The correlation is more than 96%. With PZA, it's 97% or even higher. But then with INH, it depends which gene one is analyzing. It never adds up to 100%. So that means we still have a lot of work to do.

The California National TB Center in San Francisco published this year, January or December of 2004, a so-called survival guide for clinicians.

And I know…and others from the laboratory community work diligently to make this an all inclusive guidelines, which actually can be downloaded from the Web site specified here from the National TB Center .edu. And it's worthwhile looking into these guidelines because all aspects of drug resistant TB is really nicely covered.

Shifting gears to tuberculosis complex, so now we are dealing with identification issues. As many of you may know, nucleic acid amplification, regardless of the commercially available brand, Acuprobe, and …sequencing, they detect all members of Mtb complex. At the same time, that means also that we cannot identify individual members and specify them to the final species level. So with other words, when we have nucleic acid amplification positive, then it means M. tuberculosis complex. However, we need to know what the individual species are.

And the…in Paris published three years ago, a nice proposed evolutionary pathway of TB organisms in a way comparing the data from the entire chain sequencing in M. tuberculosis versus vile-type. And they proposed in certain regions, which were lost as one that goes on with canettii on the top, then we have M. tuberculosis, then Africanum, microde, and various

level of bovis. Some of them in the meantime have now their own species status like SEAL, which is…, and Goat, which is the…

Based on this basic research data, then subsequently, again, the…people with Linda Parsons from Wadsworth Center, proposed the so-called deletion analysis assay to more rapidly identify the various species. And there we have a primary set of RD1 and RD9 and RD10, RD meaning regional deletion. And then when it's not TB, that means, first row, with all being positive. If one is not positive, then we would add two more. In the original paper, we had RD4, RD5 and RD12. Currently, we do only RD4 and RD12. If microde is in the picture, then we may do an extra revised RD4.

To convince everybody how important it is to final ID TB complex, in this table you can see our data from 2001 through 2004, and only 95% actually is M. tuberculosis. We have Africanum, almost 2%, bovis 2%, capri, a single isolate, and then bovis BCG almost 1.5%.

Some of these Africanum strains, they were isolated from patients they were never in Africa. So don't make the mistake thinking only Africanum is seen in people from Africa.

The bovis and the BCG by now, I think, it's clear how important this differentiation is. The BCG, most of these 23 isolates are isolates, which were recovered from patients suffering of bladder cancer, which were treated to stimulate their immune response intravesically against the bladder cancer with BCG, and then the BCG disseminates. And we have cases where we were able to detect the BCG out of the blood sample or a tissue or in the abscesses, and so on. So with other words, it's clear to recognize that the BCG can be detected, and it will be a mistake to have them labeled as tuberculosis or tuberculosis complex.

And this becomes now even more important to find identification. As I talked earlier with the NCCS guidelines, PZA is not recommended there any longer in the first set. So we are losing another trigger, meaning, when you have TB complex mono-resistant PZA, this is most often either M. bovis or M. bovis BCG. Although there are some strains mono-resistant to PZA, which are actually tuberculosis. But the vast majority of these mono-resistant PZA are either M bovis or bovis BCG. And since a lot of clinical laboratories are not testing any longer routinely the PZA, it becomes even more important that we do the final ID.

Another example how important it is, a couple of months ago morbidity/mortality weekly reported about the human tuberculosis caused by M bovis in New York City. And we're talking here about 35 cases with M bovis, a little bit more of an extrapulmonary than pulmonary cases.

If we wouldn't have had a mechanism in New York State requiring that the final ID is mandatory, I'm pretty sure we would not have detected all of them. Thirty-five cases is quite remarkable for not being a border state to a region where cattle TB is still prevalent. And in this particular publication, the epidemic is not yet identified what actually caused these M bovis infections, but we assume it is un-pasteurized cheese, which is imported from Mexico, and the vast majority of these bovis TB cases are actually Mexican.

Nucleic acid amplification is already approved by FDA since ten years ago for a smear positive respiratory specimen, and CDC then five years later issued the first guidelines, which are current under review. For one brand, the smear negative respiratory specimen is also FDA approved, and we are wondering why nucleic acid amplification is not more often used to confirm and diagnose TB early on.

Healthy People 2010 published January 2000, that means we have another five years to go. And I don't see how we are able to meet the Healthy People 2010 goal, which actually requires 14-14 reduced turnaround time for laboratory diagnosis. And the target would be two days for 75% of the testing. Without nucleic acid amplification, there is no way that we can meet this target.

And in New York State, for many years we had more a less voluntary to use nucleic acid amplification, and we realized, finally, that we are not getting to this target. And as of next year, 2006, the nucleic acid amplification test is mandatory in patient first time diagnosed with a smear-positivity in a respiratory specimen. And, hopefully, with this additional requirement, we may be able to meet the overall target and the second phase will be then also when the clinician has a high suspicion, even when the smear is negative, that we will then use the nucleic acid amplification as well.

In terms of errors, oftentimes people don't like to talk about their errors and they are very prominent members, alive, I would say. But already in Colorado, Burman and Reves; these are not two laboratory people. One is a clinician, one is a TB controller; they published in 2000 a nice review

about false-positive cultures for Mtb and recommendation for avoiding unnecessary treatment. What they did is they did a little research. They enrolled only studies of 100 patients, and 12 of them actually had DNA typing results, and they mentioned that the definition for false-positivity varied greatly.

But the reason why I bring this to your attention is they recognized a median false-positivity rate of 3.1%. And you can imagine that nobody in the laboratory can be happy about 3.1% false-positive error rate. And you can see in the quartile range is even broader. So there are some laboratories even with more false-positivity. And yet that doesn't mean, necessarily that everything happens in the laboratory since we have a pre-analytic, analytic, and post-analytic phase in terms of laboratory testing. If the sputum specimen was mislabeled on the TB board, then it's not the laboratory's fault. But we should not assume that all these 3.1% are somebody else's mistakes.

Leap from the Harvard Medical School already in 1994, I think that…correctly. Errors must be accepted as evidence of system flaws, not character flaws. That means no finger pointing until and unless that happens, it is unlikely that any substantial progress will be made in

reducing medical errors. So, hopefully, at some time we are able to transform this philosophy into the laboratory environment, and maybe starting a voluntary reporting of errors, so after that we could minimize these data and then the entire community could learn from and actually prevent future errors.

I will not talk a great deal about TB fingerprinting, however, just to reiterate that universal real-time genotyping is now available through the National TB Genotyping and Surveillance Network. What they do is compare…in mirror real time versus conventional RFLP. And I assume, since the vast majority of people who are actually enrolled in this conference call are public health laboratories, and public health laboratories should know that most likely this is going on in their own state.

What are the real benefits of the universal DNA fingerprinting of M. tuberculosis complex? When you ask TB controllers, the first answer is detecting false-positive cultures. The second is uncovering previously unrecognized cases of transmission, which the so-called conventional shoe leather epidemiology was unable to detect. And then lastly, they also

recognize that the fingerprinting is very good in assessing efficacy of TB control programs.

Coming back, since we dealt now with the sick people, going a step further to the infected person. And when we are talking about infected people, PPD, and tuberculin skin tests, then I have an obligation to talk about Florence Seiburt from the Henry Phipps Institute in Philadelphia, who actually produced the world standard called PPDS, and the S actually stands for standard and not for Sieburt. But what is amazing is it became the U.S. standard in '41, '52 the WHO standard, and it is still the standard in 2005.

CDC was very smart when they commissioned the report about ending…and they were able to get this report written by an esteemed panel with the Institute of Medicine. There they clearly mentioned what needs to be done if we go from the level of control to the higher-level of TB elimination and elimination was defined as one less than one case per one million population per year. Usually when we talk about case rates, it's one per 100,000. So here, we talk now about less than one case per one million population.

One of their recommendations is what really needs to be accomplished in this journey. The greatest needs in the U.S. are new diagnostic tools for the more accurate identification of individuals who are truly infected and who are also at risk of developing TB. The truly infected, I think, in 2002, we have new assays. And I will talk soon about them, to identify the truly infected and not the false-positive one which may interfere with vaccinated individuals with BCG. But we are still far away to protect who actually, from the tuberculin skin test positive individuals will come down later during lifetime with symptomatic tuberculosis.

…from San Francisco and colleagues, they wrote a nice glance at ID review in 2004 about interferon gamma assays. And it would be worthwhile for all of the listeners to get this review and to get acquainted with the issues about interferon gamma assays.

One brand, QuantiFERON-TB, is FDA approved since November 2001, and CDC guidelines published already January 2003, and they actually are in revision. And believe it or not, the next version of these guidelines will be published in a couple days, next Friday, December 16 in the morbidity/mortality weekly report, where we'll learn then all the indications and certainly also limitations of this test when to apply this

interferon-gamma assay. The other one, which is also commercially available in Europe and in Asia is the T-SPOR-TB. However, it's not FDA approved in our country.

Performing the assay, there are four phases: blood collection, and it should be…cube, incubation of blood with stimulating antigens and the QuantiFERON-gold version actually includes these more TB specific antigens, ESAT-6 and CFP-10. Then after the stimulation, one has to detect how much gamma-interferon was produced through an ELISA assay, and then ultimately the interpretation.

Since the QuantiFERON assay is an ELISA based assay in our public health laboratory here at Wadsworth Center, we actually don't do the assay in the TB laboratory, rather the HIV folks are doing this assay.

What are the pros? What are the cons? The pros compared to tuberculin skin test, it requires only one visit. So if you place a tuberculin skin test and the patient doesn't show up for the reading, your effort is already in vein. Then, second, a simple format. Thirdly, more objective than TST and, I think this is really great to have a measurable by a machine, rather than a guesstimate how many millimeters the tuberculin skin test is. And

also, blood samples are more likely to be of good quality specimens than individually placed tuberculin skin tests, where you don't know if actually the test was applied properly.

The negatives, we have first, antigens not TB specific. In rare cases, M. kansasii, sulgai, and marinum may cross-react. However, the gold version, as I said, since it contains ESAT-6 and CFP-10, the BCG at least is out of the picture. Second, we still need to set up to stimulate the lymphocytes within 12 hours. That means there is still no way to ship overnight the blood specimen. And, hopefully, in the near future, this will be resolved so it becomes like a clinical test for glucose or so, and less a issue for the public health laboratory. And thirdly, the clinical experience is still limited.

These are three cubes from the interferon gamma assay, the…version where we have a red cap with TB antigens coded. Then we have a purple cap which tests, in a way, the cell mediated immunity with a mitogen and then we have the control where we have nothing.

What are the unresolved TB lab issues? Processing sputum - we still use leftover sediment as the basis for molecular workup. Can you believe

that? Post-marketing surveillance - although we have more and more kits FDA approved, we don't have any systematic surveillance after FDA approval to see how well the system actually worked when we gather more data.

The most serious one is the TB meningitis. The sensitivity of laboratory assays still today is inversely proportional to the seriousness of the disease. With other words, we know how limited nucleic acid amplification is in HIV cases; the smears, the culture, everything doesn't perform well in these patients.

If we assume that India, with 1.8 million new cases every year, has about every year 18,000 or up to 50,000 TB meningitis cases, and we don't have a good assay to help them in protecting these serious forms of tuberculosis, and no improvement is around the corner.

This brings us to the summary and the cardinal, the king fisher and the black skimmer and the seed of…they all say the same thing. "It is health which is real wealth, not pieces of silver and gold." Quote from Gandhi.

But also being most of us here on the call we are within the public health laboratory network, we need to stress to work together with TB controllers, but also with local laboratories, which are hospital based or commercial laboratories and then even among the state public health laboratories as well.

As I mentioned, some of these Fast Track programs initiated early 90s, they are still there. It still state-of-the-art. We add newer technologies. We still shoot for the shortest turnaround time, and it's not any longer only smear-positive respiratory specimens. We also include smear-negative if a board certified pulmonary or board certified infectious disease doctor or TB controller signs off of them. But it also means that with the issue about drug resistance, we have to broaden the Fast Track programs to include suspect of drug resistant TB.

In this regard, the California State Lab and the New York State Lab, we both offer rapid molecular assays to detect…and resistance, and I actually don't know if other public health laboratories are offering this. But here, again, I think we need to tap into these resources to make sure that all TB patients, regardless where they are, have access to the latest proven technology.

With this, many of you may have seen this slide, never give up. I still feel we are in the position of the frog, and we can say "Hang on. Never give up fighting TB." And that should lead to fighting poverty, and ultimately standing up for peace on Earth. And my very last slide, happy holidays. Thank you so much.

S. Glinos Thank you, Dr. Salfinger. This is Sophia again. We will now take some questions if the operator would like to take over and let everyone know how to do that.

Coordinator Thank you.

S. Glinos Well, we do have one question here. The question is what type of controls are you using for antimicrobial susceptibility testing? Max?

Dr. Salfinger The susceptibility testing in the past we required a drug resistant strain to include with a susceptive strain to have it done at least once weekly or whenever a susceptibility test is set up, if it's not every week. And as we have false-positive cultures with a control strain and the issues about drug resistance since early 90s in the manual of clinical microbiology, it is recommended to use a susceptible strain and to do MICs. Because with

this type of approach, you recognize subtle differences better than if you are using a highly resistant mutant laboratory control strain, which you have to make a real huge error that, such a drug resistant strain becomes susceptible in your assay.

So what we are doing is in the BACTEC460, since we are still using the 460, the MGIT960 is still in evaluation here at Wadsworth. So, for instance with Ethambutol, the low concentration we test is 2.5 and dilute this further down 1.25 and .625 and the true concentration, the higher ones will be susceptible with a control strain, and the lower one will be resistant. So with other words, in these MICs, we have then always a resistant concentration, which will be read as resistant, and then the next higher one should be already susceptible. And if there is a mistake, then we have this earlier detected than if we would use actual high drug resistant mutant as a lab control.

Coordinator We do have a couple questions if you're ready for those?

Dr. Salfinger Yes, I am.

Coordinator Lillian from New York, your line is open.

D. Carb — This is David Carb from the TB lab here at New York City Department of Health. My question involves the spoligotyping service that your state provides and whether it could be utilized for the M. bovis or M. bovis BCG identification. It's my understanding from one of the experts here that there is a very specific pattern for M. bovis, and that the spoligotype, which is done on a mandatory basis on all new isolates, could, in fact, give an identification. Is this correct?

Dr. Salfinger — This is correct in terms of the M. bovis, and the M. bovis BCG there are several types, and we don't know necessarily enough information in terms of Africanum or in terms of capri. However, we need to realize since we have this network now going on, it would be really good if we could use this network or the heat kills of these strains, than really to do routinely deletion analysis or other gene analysis, which allows us with the identification like…B in combination of RT1 or so, which then gives us also a final ID.

But I agree, the bovis could be spotted on the spoligo. But although we do the spoligotyping in-house, we always confirm it with the deletion analysis because there is no paper actually published, which validated in a way this assumption. While we have validated the deletion analysis, but

the conclusion from the spoligotype that always you will be able to identify the bovis is not yet published. And therefore, we still require this final ID. But we are certainly willing to accept through the heat kill spoligotyping and then confirm by deletion analysis.

Coordinator — Our next question comes from Ken from Hawaii. Your line is open.

Ken — Hello, Dr. Salfinger. I've got a question, actually two questions. On the false-positive cases, I noticed that the 14 studies, you said that 12 included DNA typing. Was there a big difference between the false-positives that used the DNA typing versus those that didn't, and can you give us an idea of what the results were?

Dr. Salfinger — I don't have the results handy. That's why I gave the reference to the paper. But why they make this comment about DNA typing is with genotyping, they were able to confirm that it was really a laboratory error and not an assumption. And I think this was the beauty of this study that 12 out of 14 actually had supporting data that the error actually happened in the laboratory.

Now you had a second question?

Ken — Also the question about the-- You said that BCG was used in New York, and that because of this using BCG for therapy for bladder cancer, that there were cases of M. bovis infection. Is that correct in terms of what I understood?

Dr. Salfinger — I'd like to clarify this. If a patient has a particular bladder cancer, the standard gold therapy is to have instilled six week, once weekly BCG into the bladder as a local stimulation for the immune response. And, actually, the bladder goes away. And this is done with BCG. And this is well published since early 70s. And what we are missing here since BCG is not a reportable entity, we have no denominators. So we don't know in all these cases, the urologist is treating these bladder cancer patients with BCG, how often we have this adverse reaction that the BCG shows up outside of the urine wherever. And we don't know how often this is and we don't even know how many times the clinical laboratory may actually misidentify these isolates as M. tuberculosis.

Since usually the bovis BCG is weekly niacin-positive, and then some say, oh it's Acupro-positive for instance, and niacin positive. Therefore, it's TB, because the laboratory has no clue, usually, if the patient has bladder cancer or not. And that's why I think this is just another reason why we

need to have this final ID cross the country of M. tuberculosis complex. Not only the bovis in Texas and California, cross-boarder issue, or now the bovis epidemic most likely, I have to say most likely, contaminated the dairy product in particular cheese from Mexico. It's also to get a handle on the prevalence of this adverse reaction from the BCG treatment in the bladder cancer patient.

Coordinator Thank you. Our next question comes from Shelly from Alaska. Your line is open.

Shelly Yes, sir, we have two questions for you. The first question is do you repeat your susceptibility testing after three months on all your TB patients, or just those that are resistant to one of the drugs that you've used in your drug regime?

Dr. Salfinger No. As a reflex testing, we repeat it automatically. So we don't even ask for a request from TB control or from the physician. If we recognize that the sample is now more than three months in-between positive, than as a reflex, we do automatically the full panel…PZA in the BACTEC460. When something is resistant, then we go and repeat this in the arc of

proportion through the molecular…and extend to a second line in the arc of proportion as well.

Shelly — All right, sir. And our second question is you also stated that if a positive culture is still positive at four months, then you call this treatment failure. So is this on all patients or is it those who have resistance, because sometimes the plates will have just one or two colonies, even at four months, even though their positive smears have been decreasing all along?

Dr. Salfinger — The issue about four months, what it means is taken straight out of the TB treatment guidelines, so these are TB control terms. When they have a positive lab result at month four, that means the patient is considered a treatment failure, and they have to change their drug regimen according to this event.

Shelly — All right, thank you, sir.

S. Glinos — Thank you. We're sorry, at this time we have no more time for questions. If your question was not answered, you mail e-mail it to the Northeast office at neoffice@nltn.org. The speaker will answer your questions by e-mail. Again, that e-mail address is neoffice@nltn.org.

Again, I'd like to remind everyone that are listening to our program to register and complete an evaluation form by January 14th. The directions for this are on your confirmation letter and general handout. They were also e-mailed to each site rep this morning. Documenting your participation helps us to continue to bring high-quality training programs in a variety of formats. When you've completed the registration and evaluation form, you'll be able to print your CEU certificate.

That concludes our program for today. The co-sponsors of today's program would like to thank our speaker, Dr. Max Salfinger. Thank you for joining us. I hope that you will all consider joining us for future programs, and that you will make the National Laboratory Training Network your choice for laboratory training. From the Wadsworth Center in Albany, New York, this is Sophia Glinos. Have a nice day.