FTS-CDC-PHPPO

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Coordinator — All participants will be able to listen only until the question and answer portion of the conference. This conference is being recorded. If anyone has any objections, you may disconnect at this time. I'd like to introduce the host, Mr. Randy Graham.

R. Graham — Good afternoon and welcome. This is Randy Graham. I am the state laboratory training coordinator for the Minnesota Department of Public Health Laboratory in Minneapolis, Minnesota. Welcome to part two of our antibacterial susceptibility testing 2005 teleconference series. Today's topic is detecting resistance in gram negative bacteria, including ESBL issues. The teleconference is sponsored by the following institutions: The National Laboratory Training Network, the Minnesota Department of Public Health Laboratory, the North Dakota Public Health Laboratory, the University of Iowa Hygienic Laboratory, the Nebraska Public Health Laboratory, the Michigan Department of Community Health and the

Wisconsin State Laboratory of Hygiene. The National Laboratory Training Network is a training outreach program sponsored by the association of public health laboratories and the centers for disease control and prevention.

Here are a few program notes. You are participating on a listen-only line. You can only hear us; we cannot hear you. If time permits, we'll open up the phone lines at the conclusion for questions. After the program, each participant needs to complete an online evaluation form. Documenting your participation helps us to continue to bring high quality training programs in a variety of formats. In addition, all participants will receive a certificate of attendance for participation in each teleconference. Your site representative will provide you with a certificate and instructions regarding the online evaluation. You have until March 24th to complete this process.

From the plains of Nebraska to the shores of the Great Lakes, we have over 200 sites listening to this conference. It is my pleasure to introduce the speaker for this series, Ms. Janet Hindler. Janet is a senior specialist in clinical microbiology for the division of laboratory medicine at UCLA Medical Center in Los Angeles, California. Ms. Hindler is well known

throughout the United States and internationally for her extensive work in the field of antimicrobial susceptibility testing. She's working as a consultant with the Association of Public Health Laboratories to develop and conduct training on antimicrobial susceptibility testing. I'll turn it over to Ms. Hindler.

J. Hindler Thank you, Randy and participants for once again tuning in this series. The title of today's presentation is detecting resistance gram negative bacteria, to include a discussion of ESBLs. I'm assuming all of you are either looking at a copy of this on a handout or a PowerPoint presentation. I'll go over the slides and make comments. I've also included a reference list and may refer to them, and will highlight certain references for you.

At this point on slide two, let's just review the objectives for this presentation. We're talking about gaining information as related to gram negative bacteria. By the end of the presentation, you should be able to discuss reliable methods for detecting resistance among commonly encountered species of gram negative bacteria, explain how to implement current CLSI and a micro susceptibility testing and reporting recommendations, and I'm assuming most of you tuned in to the first lecture when we discussed the CLSI standards, formerly known as the

NCCLS standards for … microbial susceptibility testing, list results obtained from testing patient … that should be verified prior to reporting these as part of the quality assessment program.

On the next side, we'll start with the introductory information for what we're going to talk about first, and this is related to beta lactamases and gram negative bacteria or rods.

Slide four is a chart showing the evolution of beta lactamases. We're primarily talking about the tem type and SHB type beta lactamases, the common ones found in the gram negative bacteria. If we look at this chronology, you can see the 1963 ampacillin was introduced for clinical use. In 1965, we saw the first inner bacteriasia producing a beta lactamase that would destroy ampacillin. The tem one beta lactamase was described in e-coli and certain salmonella species. Then by the 1970s, we saw the tem one beta lactamase report in 28 different gram negative species. The extended spectrum beta lactams or extended spectrum cephalosporins were introduced in the late 70s, early 80s. As early as 1983, we started seeing ESBLs in Europe, and these destroy extended spectrum beta lactam agents. In 1988, we started seeing ESBLs in the United States, and in 2000, we had over 130 different types of ESBLs described worldwide, and

to date in 2005, this number has increased approximately 200 different types of ESBL enzymes.

As you can see, the complexity of beta lactamases among the … is something of concern and complicates our ability to detect resistance to beta lactam agents among the …

Slide five, let's talk more about these beta lactamases and the tem one beta lactamase that's coded for by the beta lactamase or BLA tem one gene confers ampicillin resistance in e-coli. So most of our ampicillin resistance e-coli are producing this tem one beta lactamase. The ampicillin resistance in … influence, those strains that are beta lactamase positive most likely will have this tem one beta lactamase. Similarly, beta lactamase producing nyceria gonorrhea is likely due to the presence or production of this tem one beta lactamase. For ampicillin resistance in … , the likely beta lactamase causing that is the SHB1 and this is coded for by the beta lactamase SHB1 gene.

Now, recently in the 1980s, these basic tem one and SHB genes underwent small point mutations. So the resultant genes code for extended spectrum beta lactamases. So the evolution of the … is from the common beta

lactamases that was described in the early 60s and into the 70s, coded for resistance to the narrow spectrum beta lactam agents.

Slide six, we have a few definitions. When we talk about narrow spectrum beta lactam agents, these are those drugs that are active against either gram negative or positive bacteria. An example is penicillin that's only active against gram positive bacteria.

In contrast, we have the broad spectrum beta lactam agents, and these are active against gram negative and positive bacteria. These would include ampacillin and the first generation cephalosporins.

Slide seven, we have the extended spectrum beta lactam agents. These have enhanced activity against gram negative and some gram positive bacteria, and now we're talking about the third and fourth generation cephalosporins, the carboxy or ureido penicillin, basically your piperacillin, mesocillin and ticarcillin, and these are the extended spectrum beta lactam agents. As we pointed out last time, we mentioned the glossary found in our NTCLS or CLSI M100 tables, and in that you could find which agents are included, and the cost designated carboxy or ureido penicillins.

The extended spectrum beta lactamases are enzymes that, produced by gram negative rods, that destroy certain extended spectrum beta lactam agents, and these include the third generation cephalosporins. So you can kind of understand the terms we use to describe these different types of beta lactamases.

Slide eight, I've included a picture of our educational CD Rom we developed with CDC in 2002. We're not going to talk in detail about methods for detecting some of the resistances that we'll mention in today's presentation, but the extreme details for performing these tests are found on this CD Rom. If you don't have a copy of this CD Rom in your lab, you can go to the URL listed on this slide. There is place there to order this CD Rom free from CDC. I encourage you to do that. It's valuable information, not only about gram negative organisms but all organisms you might be testing for susceptibility in your lab.

If you go to slide nine, there is a photo of one page from the CD Rom, and this shows a schematic or flow chart type of diagram describing the abundance of beta lactamases or the variety of beta lactamases found among the … This page has a number of these boxes. You click on it and

it tells you in detail what that beta lactamas characteristics are. If you want to learn more, I encourage you to go to this page, click on these boxes and you'll learn more than you ever wanted to know about beta lactamases among the …

The point I'm trying to make is beta lactamases among … are extremely complex, and that's why we have to have special test and reporting procedures to test some of these. I might also mention that many of us are doing beta lactamase testing in our laboratory, for example on homopholis influenza. Most of these gram negative organisms that product beta lactamases are going to give a positive beta lactamases reaction if we were going to use a nitro … test or another test. The problem is, having a positive result for beta lactamas among these organisms isn't really going to help give us any information that could help guide the physician in using specific beta lactam agents and treating infections caused by gram negative organisms or bacteria.

Therefore, it's totally inappropriate to do a conventional type data lactamase test such as your … nitrocephan on the inner bactracia because we won't get any useful information to guide physicians in choosing an antimicrobial agent.

Next slide. We talked about the inner bactracia. We're going to discuss extended spectrum beta lactamases. We're going to discuss the AMC beta lactamases. We'll also talk about detecting resistance among salmonella …

Slide 11, this is an excerpt from the Sanford guide, which is a publication the physicians often refer to because it includes recommendations for drugs that can be used for treating infections caused by various bacteria. So I've included just a snapshot on this slide, the recommendations for first choice agents to be used in treating infections caused by … I want to show you that when we talk … we see at the top of the list, a third generation cephalosporins. Also, quinalin, carbetenim, urda, immie, or … When you go back to the enter bacter such as enter bacter Quaque, enter bacter erogenous, we don't have a listing for the third generation cephalosporins. We see the carbetemims and then a recommendation or suggestion for possibility using an extended spectrum penicillin plus the …

We're saying that … and actually e-coli would fall into a similar category that third generate cephalosporins are still choices to use in treating infections caused by … e-coli, you'd see similar recommendations.

That's why now we see ESBL producing organisms that confer resistance to the third generation cephalosporins, it's really important. Previously, the e-coli and … were all susceptible to third generation cephalosporins, and it was a given that these agents could be used. But now when we have e-coli resistant to a third generation cephalosporin by virtue of production of an ESBL, this is a surprise. So it's important we be able to detect this type of resistance due to the ESBLs and make sure we communicate this to physicians so they're advised not to use the primary drug of choice for treating infections caused by the … e-coli. This is not as significant of a concern in the … where the third generation cephalosporins aren't considered first choice agents for treating infections caused by the …

Slide 12, why is it important to do these special tests described in this CLSI document for ESBLs and following the complex reporting rules? We know that we may have in vitro test results that might be misleading. We may see a susceptible result for a third generation cephalosporin in

vitro, but we know it isn't going to be effective in treating an infection caused by an ESBL producing organisms.

We also know there are many different types of ESBL enzymes with various susceptibility profiles, and I already mentioned there are about 200 different types of ESBL enzymes, and they may result in different susceptibility profiles among the beta lactams on our panel. We know for a fact there are treatment failures if a patient that has bactaremia due to an ESBL producing organism is treated with a third generation cephalosporin. We also know the ESBL producing organism can cause no … outbreaks. These points illustrate why it's very important for us to look for ESBLs and report them appropriately in our laboratory reports.

Slide 13, I've included data that has actually demonstrated this with real patients. This was a study done by Patterson and colleagues a few years ago where they looked at patients with serious ESBL infections treated with third generation cephalosporins because they tested susceptible. So among the 28 patients in this study, all had islets that were susceptible to a third generation cephalosporin, so that was what was used.

But if you look at the total results in the second column, 54% of the 28 patients failed therapy with a third generation cephalosporin even though it had tested susceptible. So we're seeing there is something wrong with our supporting susceptible results for ESBL producing islets because the results for a third generation cephalosporin does not translate into effectiveness of that agent in treating a serious infection caused by ESBL producers.

Therefore, we have to look for these using special testing. On slide 14, there is a summary of the testing we can do to identify organisms that are ESBL producers. According to our current CLSI documents, the organism in which we're going to look for ESBLs include e-coli … and most recently, prodias marablas has been added. A screen tested described where we're looking for decreased susceptibility to extend a spectrum data lactam agents, indicator agents, and if we have an islet suspicious for ESBL production, we're going to do a the phenotypic confirmatory test where the actual test methodology involves testing a beta lactam with and without a beta lactamase inhibitor, with the understanding that if it's a ESBL producer, the beta lactamase inhibitor is going to restore the activity as the beta lactam.

Slide 15, I mentioned currently, in the most recent CLSI standards, prodias marablas was added as an organism in which we should look for ESBLs. But it's important to note that ESBL producing prodias marablas are very uncommon in the U.S. today. They've represented significant problems in some other locations, however. Later, I'll tell you the strategy we can use for testing prodias marablas for ESBLs.

Slide 16, let's review the CLSI report rule for islets that are confirmed to product ESBLs. The quote listed states, "The strains of … species, e-coli and prodias marablas that produce ESBLs may be clinically resistant to therapeutic penicillins, cephalosporins or … despite apparent in vitro susceptibility to these agents." What does this comment actually mean? The message for us in the laboratory is that if we encounter an ESBL producing strain, we have to report these as resistant to all penicillins, cephalosporins and … Again, too, to know which particular drugs fall into the penicillin or cephalosporin class, we can refer to our CLSI glossary.

Slide 17, let's define which beta lactam results would be resistant once we identify an islet as being an ESBL producer. The penicillins, all which include amp, carban, mesocillin, … the cephalosporins, which would

include cephalexin, cefamandole, cefuroxime, cephotaxime, ceftriaxone, ceftazidine and cefepine, the mono-bactam …

Slide 18, there are some beta lactams that we're not going to edit as resistant if they do test susceptible in these ESBL producing strains. These would include the cephamicins such as cefoxitine, cefotetan or cefmetizol, and then the beta lactam inhibitor combination. The reason is these agents are not as rapidly hydrolyzed or inactivated by the ESBL enzymes like the drugs listed on the previous slide. That's why we're not now editing to resistant the cephamicins or the beta lactam inhibitor combinations.

Slide 17, this is the listing of the screening points we use to identify whether or not an e-coli … prodias marablas is indeed suspicious for ESBL production. These break points are different than the standard break points used to interpret results for these particular agents among the … Here you can see cefadoxine, cephotaxime, ceftriaxone, … have unique disk zone interpretive criteria or brave points that would confirm that the islet is suspicious for ESBL production and corresponding MIC brave points, as well.

I have cefadoxine, cephtaz and cephotaxime asterisks is because for prodias marablas, it's only three agents that are appropriate to use as screening agents. Also for prodias marablas, the break point for cephadoxine by the MIC methodology is different than that for e-coli and … being an MIC greater than one would indicate if prodias marablas was suspicious for ESBL production.

Slide 20, here's an example of a report you might issue from your lab when you encounter that clebciela this is suspicious for ESBL production, and this particular scenario was due to a cephadoxine MIC of 16.

We've listed a number of agents and have a comment on the bottom that we're not releasing, at this time we're suppressing results for cephatim and cephotaxime because they tested susceptible. We'll do the … test. If this organism turns out to be a ESBL producer, we're going to have to edit the cephatim and cephotaxime susceptible results to resistant. We want to avoid reporting them as susceptible today and then do the confirmatory test and if they are ESBL producers, we'll have to go back and amend those results to resistant. Therefore, we could report all those drugs that aren't going to be effected by confirming this islet as being an ESBL producer. You can see those right here. In our laboratory, we also include

a comment saying that this is suspicious for ESBL production, confirmatory tests are pending.

Slide 21, we describe the … confirmatory test where we have cephotaxime … by a variety of different methods, and we're looking for the … to restore the activity of the cephotaxime or cephtazime or both. We have specific quality control recommendations for this ESBL … confirmatory test, as well. These quality control recommendations are listed in the specific ESBL tables found in the CLSI document.

If you go to slide 22, this is an example of one of the methods that can be used for the … confirmatory test, this is the disk diffusion method. At about 5:00 and 7:00 o'clock, you see cephotaxime and cephtazime alone. Then at about 4:00 and 8:00 o'clock, you see these agents combine with … acid. Let's look at the cephotaxime, the zone diameter for cephotaxime alone is very small, however the zone diameter of cephotaxime when combined with the beta lactamase inhibitor, the zone is substantially larger. In order to indicate that this is a positive … confirmatory test, we want to see the zone diameter with the acid to be 5mm or more greater than the zone diameter for the cephotaxime alone. In this case, you can see that is the case and this is positive.

If we look at the cephtazime, however, the zone diameter for cephtazime alone isn't much different than that for cephtazime … acid. So this would not be a positive test for ESBL production if we were just to use cephtazime pair. However, to confirm a positive result, we only need to see one pair be positive. In this example, because the cephotaxime pair is positive for ESBL production, we can confirm this islet is an ESBL producer.

Slide 23, here you can do the ESBL confirmatory test by the e-test methodology as well, by other MIC methods, too. On the e-test, on one end of their strip, you see cephtazime, on the other end you see cephtazime with … acid. Cephtazime alone does not inhibit the growth of the organism. But combined with … acid, you do see a lip. The MIC goes from greater than 32 for cephtazime alone, down to about .75 with the … acid which is restoring the activity of the cephtazime. What we're looking for as a positive reaction in the MIC test, a three two fold delusion drop in the MIC, and this particular example definitely shows a greater than three two fold delusion drop in the MIC when combined with … acid. So this is a positive confirmatory test, as well.

For those of you using the Microscan system, there is an ESBL panel on the Microscan system that you can use for the confirmatory test. On the old Vitech, some of the cards have an ESBL test incorporated right on the card. So in this situation, you do not have to do the screen test first because you're doing the confirmatory test, at the same time you're setting up susceptibilities on that islet. For the Vitech two, the advanced expert is part of the Vitech two that does actually inform you that the islet is an ESBL producer.

Slide 24, we see here the final report of this islet that is an ESBL producer, so now we can go ahead and report cephapime and cephotaxime, but because this is an ESBL producer and those agents are cephalosporins, we're going to edit those susceptible results to resistant. When you edit the results to resistant, it's recommended not to report the original MIC—for example, for cephapime it was two, and if a physician were to see an MIC of two that normally is susceptible with an interpretation of R, that might be questioned. Therefore, when you edit susceptible results to resistant and you're using an MIC methodology, do not report the MIC as well.

Here, you finally see we added a comment to this report as well. confirmatory test for this … indicate unusual resistance, extend a spectrum beta lactamases infectious disease consult suggested.

Let's go to slide 25 which reflects an islet that was suspicious for ESBL production by virtue of the screen test. However, when the … confirmatory test was performed, it's not positive. You can see there is virtually no difference between the cephotaxime zone diameter and the cephotaxime with … acid or cephotaxime and cephotaxime … acid. Here we're saying this islet was suspicious for ESBL production, but that was not due to the presence of an ESBL enzyme. There is another mechanism of resistance that is conferring resistance to the cephotaxime, cephtazime and other indicator drugs.

In this scenario, you do not edit results and that is illustrated in slide 26 where I'm showing you report of an e-coli that would have these results for their ESBL test and some other results you're likely to see when this type of phenomena is encountered. It's likely the mechanism here is that this e-coli is an AMC producer, producing high quantities of AMC that would cause cephotaxime and cephtazime to show a resistant result.

Here, too, we added a comment after we finished this testing. Confirmatory tests for this e-coli indicate unusual resistance, but not due to ESBLs. I'm not advocating you all have to put these comments on the report, but you might want to consider doing it to help physicians better understand the message you're trying to convey with the report.

I might mention, also, if you go back to slide 24, I failed to say what drugs might be used in treating infections caused by this organism. This particular example, the islet is susceptible to … If the organism were susceptible to other drug … they can certainly be used. The dilemma is that many ESBL producers are resistant to other drug classes as well. This scenario is probably over-susceptible compared to many of the ESBL producing strains we see.

As we said, we're not going to edit results for … cephoxitin, so conceivably, those agents could be used as well. It's unlikely, however, that physicians would use either cephoxitin or … for treating serious ESBL infections because there is just not an abundance of documentation and literature that these drugs would be effective. So the likely beta lactam a physician would use for treating an ESBL producing organism

causing an infection would be imepenum. To date, it's my understanding that imepenum resistance has not been reported.

Slide 27 asks another question about the ESBL test, that being if the ESBL test is positive, must we do the confirmatory test prior to reporting an islet as an ESBL producer. Some labs feel you just have to do the screen tests. I'd say there are situations that might be appropriate. We're saying only in those situations, if the patient previously had an islet confirmed to be an ESBL producer, you see that islet again being cultured and … profile as identical to the first one, those situations, I feel if you're confident it's the same islet, it's not necessary to repeat the ESBL confirmatory test.

The other situation would be if you have an endemic strain in your institution that has a specific susceptibility profile and you're confident that's the strain you're seeing in multiple patients, it may not be necessary to do the phenotypic confirmatory on all of these islets.

In all these situations, before you report an islet or assume an islet is an ESBL producer, I think you have to do more than a screen test, and that being going to the phenotypic confirmatory test.

Slide 28. What about testing reporting for ESBLs on urine islets? In our CLSI document, it says the decision to perform ESBL screening test on all urine islets should be made on an institutional basis. The rationale behind this is that the concentration of beta lactams in the bladder is very high and overcomes hydrolysis by ESBL enzymes. So when there is so much beta lactam there that even if you have ESBL enzymes being produced by the organism, there is not going to be enough to destroy all of that beta lactam that would be present in the bladder.

To concerns, identifying urine from patients with acute cystitis versus other urinary type infections such as urosepsis. This concept is certainly true for patients with acute cystitis where the infection is confined to the bladder, but it would not be appropriate if it represents isolation of an organism that is representative as a systemic infection.

Also, patients that have islets from the urine as well as other sites, the same bacterium. For example, if we call the islet in the blood an ESBL producer and we know the islet in the urine is the same organism but we don't do ESBL testing on it, we may send a different susceptibility result suggesting to the physician this might be two different organisms. So we have to be careful about that.

Finally, infection control issues, if your institution wants to put all of these ESBL patients in contact isolation, you'd want to identify these organisms, no matter what the site.

Slide 29, will the CLSI confirmatory test detect all ESBL producing … the answer is no. Some islets may have ESBLs with other resistance mechanisms that may mask a positive ESBL phenotypic confirmatory test. There are strains that have been reported to have multiple ESBLs or combinations of ESBL and C-beta lactamases or combinations of ESBL and poor mutations, so this would render the organism resistant.

Now the ESBLs occur in species other the e-coli, … and prodias marablas, and this time CLSI does not address these because we do not have convincing evidence suggesting that the current phenotypic confirmatory tests are reliable for the other organisms that might harbor ESBLs.

Slide 30, what are the CLSI efforts currently underway to try to resolve this dilemma we're all having with detecting and reporting ESBLs. The CLSI has a very active working group, looking at the best way to determine or detect … with all different types of ESBLs or other beta

lactam resistance mechanisms. For example, one aspect being looked at as possibly lowering the third generation cephalosporin break points. For example, the current break point for … is less than or equal to eight. The proposal is to reduce this to less than or equal than one or two with the hope that by lowering the break points, we would be able to detect all different types of beta lactam resistance mechanisms, not just those due to ESBL producers. So this is something we'll hear more about in the future, and there will probably be some modified recommendations to doing susceptibility testing with the third generation cephalosporins and other beta lactam agents as well.

Slide 31. Some of you in your facilities may be asked how much ELBL producing islets are you seeing. We've extracted our data from UCLA in patients from 2000 and 2004 and looked at all of the islets that had ESBL tests performed. We did this because some laboratories use cephtazadine resistance as a marker for ESBL production because cephtazadine is a drug that will often look resistant if you have an ESBL producer. We looked at using cephtazadine resistors as a marker for ESBL production and also the actual results derived from doing the ESBL confirmatory test.

Among the e-coli, we had 804 islets, and 9.8% were cephtazadine resistant, but only 4.3% were only ESBL producers. So by using cephtazadine resistance as a marker for ESBL production e-coli may overestimate the true incidence of ESBL producers.

In contrast, if you look at the 555 … we tested, 14.1% were cephtazadine resistant, and 12.6% were positive with the ESBL phenotypic confirmatory test. So it looks the cephtazadine resistance marker can be fairly reliable in predicting whether or not that … is indeed an ESBL producer.

If you go to the next slide, it's summarizing the strategy we can use for looking for ESBLs in e-coli … and a comment about protius perform the screen test, especially islets from normally sterile sites. Any of you out there doing blood cultures and CSF cultures and finding … e-coli or prodias marablas, definitely you should be doing the ESBL testing. As I showed you, failure to identify these organisms as being ESBL producers and those patients being treated with a drug susceptible such as a third generation cephalosporin that is reported as susceptible because it's not edited to resist and because that ESBL is not explored. Definitely, we know patients can fail therapy if we fail to identify ESBL producers

among those islets causing serious infections. Perform the confirmatory test or send it to a reference lab if your volume is so low that it doesn't warrant you to keep these materials on your premises.

I have a comment about prodias marablas. The current recommendation is to perform that screen and confirmatory test primarily on islets from sterile body sites. This relates to the very low incidents of ESBL production and prodias marablas, and we didn't feel it was worthwhile suggesting to laboratories to test all of these because it wouldn't be a most appropriate use of laboratory resources.

Let's move to AMC beta lactamases. These are distinct from the ESBLs. This is probably a good time to point out a table that we've included in your handout, in the back portion, not the PowerPoint size. This is on page two. If you didn't print it, you can do it later. Basically, this chart compares and contrasts the ESBL and AMC beta lactamases. I'm not going over all the details of these, we've already described in detail the characteristics of ESBLS, and we'll do something similar for the AMC beta lactamases. I wanted to make sure you understood we included that table in your handbook.

Slide 33, we see AMC beta lactamase. There are two ways they are described. One relates to induction of AMC beta lactamase product, the other relates to selection of AMC producing resistant mutants. I might mention that AMC is a gene that codes for an AMC beta lactamase.

What is meant by induction of this AMC beta lactamase? Once again, stop thinking anything about ESBLs because this is a totally distinct type of beta lactamase. Exposure of an organism to a beta lactam that acts as an inducer can stimulate production of the AMC lactamase. We're saying there are many gram negative organisms that have this AMC gene and the ability to produce this AMC beta lactamase, but they're not going to waste their energy to produce this AMC beta lactamase unless the organism is in danger of being killed by beta lactam agents.

So when we expose it to this beta lactam agent, this is going to stimulate the organism to produce higher concentrations of this AMC beta lactamase that would result in resistance among a number of agents on our test panel. This AMC gene is usually on the chromosome. It's characteristic of virtually all strains of certain species, and this context of inducing the beta lactamase is a reversible phenomenon.

Slide 35, should we do any special testing for inducible beta lactamases? The answer is no because induction is an in vitro phenomenon and really has no clinical impact unless the therapy includes a combination of the strong inducer with a third generation cephalosporin. It's very unlikely that the physician actually prescribes two such similar agents.

Slide 36, there is a listing of those beta lactam agents more likely to stimulate inducible AMC beta lactamase. Cephomicines are at the tope of the list, cephocephoxitine is an extremely good inducer of this AMC beta lactamase. When you go to the bottom list, you're seeing third and fourth generation cephalosporins. So they don't have significant abilities to induce this AMC beta lactamase.

Slide 37, you've probably seen this elsewhere. It represents the beta lactamase induction test and the organism tested here is … You can see, to do this test, we placed the … the inducer drug adjacent to the cephotaxime disk, which is the indicator drug. On the left side of the zone for cephotaxime, there is really no distortion of the zone because there are only cephotaxime molecules in this area. However, between the cephotaxime and cephoxitin disks, we have the fusion of both of those drugs in an area where those organisms that are exposes to the cephoxitin

are going to hype up their production of this beta lactamase that will destroy the cephotaxime in the area where you see the flattening of the zone. So this is a positive reaction for the presence of an inducible AMC beta lactamase.

The next slide, we've just listed the organisms that produce inducible AMC beta lactamases as quite a variety of gram negative organisms commonly encountered in our laboratories. … providence and so forth.

Slide 39, I just mentioned here that e-coli and … not on the list so let's talk about AMC and these species. AMC is presented a little differently as compared to other enter-bacteria … so forth. These AMC beta lactamase is produced in minimal amounts in e-coli … not inducible so you can't induce greater quantities of AMC beta lactamase in e-coli and … sometimes there is a slight increase production on its own which can result in resistance to ampacillin and first generation cephalosporin. So it doesn't have a very broad spectrum of activity.

The problem is this AMC gene can be transferred from the chromosome to a plasmid. In those situations, the AMC beta lactamase can be hyper-produced. The result here is you see a resistant to a third generation

cephalosporin, cephomycines, … usually the carboxy and ureido penicillins. So a number of agents are infected by this AMC beta lactamase that ultimately resides on a … among the e-coli and …

We talked about the inducible beta lactamases. Now we'll talk about the second concept related to AMC beta lactamaces, and that's selection of resistant mutants. What does this mean? We have that AMC gene and that can spontaneously mutate as the organisms divide. So as they divide, you may have them undergo mutations, and if this happens to be a mutation the AMC gene, this can result in a greater degree of resistance to numerous broader spectrum beta lactam agents.

This phenomena is independent of antibiotic exposure and can occur both in vitro and in vivo, and in contrast to the inducer beta lactamases, once you have an organism that has mutated the AMC gene to produce large quantities of beta lactamases is irreversible. The frequency of these resistant mutants are at one in ten to the sixth and one in ten to the A cells. Normally, if you have this one resistant cell among millions of susceptible cells, this one won't survive. It will continuously produce large amounts of AMC beta lactamase to confer resistance to third generation cephalosporins, … and usually the carboxy and ureido penicillin.

Slide 41, the problem here is that one resistant mutant cell can be selected out if the population of cells is exposed to an anti-microbial agent to which they are resistant. So if you wipe out all the susceptible organisms and only that one resistant cell remains, that one can start multiplying and you ultimately have a population of organisms as totally resistant.

The frequency and selection of these resistant mutants may vary by species. This is very common among the … erogenous, but much less common among the … some may notice in your labs, you often see resistance to third generation cephalosporins among the … erogenous, but it doesn't happen nearly as often among the … and the explanation is what we just described. This resistant mutant will continuously produce the AMC beta lactamase, and that cell will be continually resistant. This concept is not reversible.

Should we do any special testing for these beta lactam resistant mutants? No, because physicians are educated that using third generation cephalosporins, … usually carboxy and ureido penicillins can select that resistant mutant among those species where this can happen. This reflects the recommendations in the Sanford guide where we don't see third

generation cephalosporins recommended for treating infections caused by the … so forth.

If we have many resistant mutant cells present in our test population, they should test resistant. Then we have to heed the CLSI rule for retesting to see if resistance has emerged in an islet that was previously susceptible, and consider adding a comment to that effect.

On slide 43, I've included the exact verbiage of what's in our CLSI document related to retesting islets that are likely to develop resistance over therapy if that organism persists. That quote is resistance may develop in three to four days for … with third generation cephalosporins, phedomonas originosa with all drugs. This is the rule to help us decide how often we need to retest similar islets from patients who previously had that islet tested from a previous specimen.

Some believe that third generation cephalosporin should not be used for … ever, so what are reporting options when these islets do test susceptible to the third generation cephalosporin? One option would be not to do anything, just report susceptible for the third generation cephalosporin without any comment. I mentioned this whole area is controversial and

that's why it's not mandated by CLSI, for example, to edit all third generation cephalosporins to resistant among the enter bactracia.

We could edit susceptible to resistant results for third generation cephalosporins. We could add a comment such as due to high likelihood of selecting resistant mutants, third generation cephalosporins may not be effected for … despite a susceptible result in vitro. The problem here is that … treated with a third generation cephalosporin, we might likely select out a resistant mutant.

This next slide represents the way we add comment to report when we encounter any of these organisms that can have mutations among their AMC beta lactamases. This relates to all gram negative organisms we include on our non-fermentative or gram negative panels. In this example, we are showing a susceptible result for cephotaxime, but we do include a comment. Combination therapy, such as the beta lactam imunoglycocide should be considered for …

Slide 46, this is an example of an islet we encountered in our lab several years ago, from a patient with traumatic meningitis. He had fallen out of a tree, had to have neuro-surgery and then got infected as CSF got infected

with … they were treating with a third generation cephalosporin and imunoglycocide, and the reason for the third generation cephalosporin because it has very good penetration across the blood brain barrier.

The first islet was highly susceptible to the third generation cephalosporin … however, the follow up culture in three days, the patient was still sick, the cultures were still positive. When the islet was still positive, you could see a much smaller zone around the cephotaxime and colonies within the zone. We're seeing that the islet previously susceptible to cephotaxime has now shown emergence resistance to this agent, those little colonies within the zone. I can't remember if we talked about this, but there is a description of this in great detail on our CD rom.

Let's move on to salmonella and shigilla, and there are a couple rules in terms of testing reporting on these. Slide 49, this is a direct excerpt from our CLSI document. "For fecal islets of salmonella and shigilla, only ampicillin, aquinaloan and trimsulfa should be test and reported routinely. In addition, … third generation cephalosporin should be tested and reported for extra intestinal islets of salmonella." I've heard labs ask if it's really essential to test for … my answer is no, unless your physicians need those results or if the islet is resistant to all other agents it might use.

Slide 50 is an example of a … report where it was resistant to amp and trimsulfa and susceptible for … But what if this happens to be from a pediatric patient, which is likely to happen, and if it were susceptible to the … in the pediatric patient. What are the options? In the Sanford guide, it says cephtriaxone, and the reason is because at that time, cephixime, which is an oral broader spectrum cephalosporin is an agent that can be used under these circumstances and has proven to be effective. Here, too, it is important to understand what additional tests we might need to perform if the organism were resistant to the primary agent we're suggesting be tested.

Slide 51 is another phenomena that has recently been described in our CLSI document related to salmonella and the … "For … susceptible strains of salmonella, the test resistant … may be associated with clinical failure or delayed response in … treated patients with extra intestinal salmonellosis. Extra intestinal islets of salmonella should also be tested for resistance in … acid and islets that test susceptible to … resistant to … acid. The physician should be informed if the islet may not be eradicated by the quintillion therapy. A consultation with an infectious disease practitioner is recommended."

What we mean is if you have a salmonella and it tests susceptible to … we need to do an … test and we're only talking about islets from sterile body types, not fecal islets. In order to find out if this islet might have reduced susceptibility to cypro, we want to test a … then report results from that.

Slide 53, we highlighted why we had to do this. Here you can see islets that have cypro … MICs in the first column of .06 or less. These would be interpreted as susceptible according to the CLSI interpretive criteria. It's unlikely these islets would have a mutation. These islets, when subjected to this … test is going to test susceptible.

By the way, … acid is an older quinalone, but it does a great job in taking out certain resistance mechanisms. That's why it's suggested if you're going to do arbitration using a … acid for that purpose would be reasonable.

If the … were .12 to one, this would be interpreted as susceptible, however some of these strains have been shown to have a single mutation towards quinalone resistant, and by retesting those islets on successive days, it's

not unlikely or uncommon for these organisms to develop true or high level … or other resistants.

If you have an islet with an MIC2, that would be intermediate. There is nothing more you can do. If you had an islet of four or greater, this would be interpreted as resistant to cypro. It's likely there is a double mutation here and they would test resistant to … acid as well. Similarly, with an intermediate result.

The problem with this is that those patients that have islets with cypro … MITs at .12 to one that would be interpreted as susceptible that have that single mutation, there have been documented cases where these cases with extra intestinal infection with salmonella may fail quinalone therapy. That's why we're saying try to detect this subtle decrease in susceptibility to … and communicate that to the physician. Again, only talking about testing islets in this manner from sterile body sites.

As an example, you see the islet that does test susceptible to … is resistant, we're suggesting at a comment, this islet has reduced susceptibility to … patient may not respond to treatment, ID consult is suggested. Because this is a rather uncommon concept, we feel it's

appropriate for a physician to get guidance from an infectious disease practitioner who could study the literature if he's not familiar with it, to try and best advise the attending physician as to how to manage the patient.

Slide 55, let's look at a strategy for salmonella and shigilla. For fecal islets, we're saying report … only. For salmonella, test fecal islets when requested. If salmonella patient is asymptomatic or illness is mild, anamicrobe therapy is often not indicated. So some labs don't do routine susceptibility on salmonella from rectal swaps or fecal sources because these are often self-limiting and they do not want to encourage the physicians to treat conditions likely to resolve themselves. However, if it was severe gastroenteritis, testing would be warranted because the patient would probably be prescribed therapy. To test … acid on extra intestinal islets from blood of salmonella that are sypro-suspectible.

Let's move on to … on slide 57, this is a revision of previous tables ones we had in our CLSI documents, suggesting which drugs are appropriate to test and report against various organism groups. What I'm point to is that for the dis-difusion method, the only non enter bactracia listed in this call … phedomonus orginosa and other non enter bactracia as the header for the MIC testing table one.

However, for both the disk and the MIC document, we have added separate recommendations for testing against the … we should all be following these.

If you go further on slide 59, there is a comment that says the phedomonus originosa and other non enter bactracia, it describes which other organisms are included. Basically, at this point, this would include phedomonus species, non-facetious glucose non-permitting gram negative rods, with the exception of … monus because we have specific drug report recommendations for these particular non enter bactracia groups. But other non enter bactracia that can be reliably tested with the MIC method but not the dis-diffusion method include phedomonus species and other non-fastidious glucose non-permitting gram negative rods. Bottom line, by dis-diffusion, the other non enter bactracia that can be reliably tested are originosa and … But also, some drugs can be reliably tested by the MIC method against … but can't be reliably tested against those methods with the dis-diffusion method, and those are highlighted in the respective tables from either the disk or the MIC document.

Table one, slide 60, there are some drugs listed in the MIC document under the pseudomonas originosa and non enter bactracia column that are not there for phedomonus originosa. Those include ticar … cephotaxime, ceftriaxone, chloro and tetracycline. Again, these are not indicated through team testing against phedomonus orginosa but may be appropriate for other species. But on occasion, some of these may be appropriate for testing on phedomonus orginosa, but not likely.

Slide 61, this is out of the Sanford guide, the drugs recommended, the anti phedomonal agents, the primary agents being ticar, pipper, ceftazidime, … they also indicate that a comment that a beta lactam is above … cipro for serious infections caused by phedomonus originosa. So a serious phedomonus infections is going to be treated with multiple antimicrobial agents, in contrast for uncomplicated urinary tract infections, a single drug usually is effective, and the most likely drug prescribed, providing it's active, is ciprofloxacin because that can be prescribed by the oral rep.

The only comment we have related to phedomonus or the non enter bactracia is in slide 62, I've reproduced that right from the CLSI document. This is a therapy related comment stating that originosa infections and … patients and serious infections in other patients should

be treated with maximum doses of the selected anti-pseudomonal penicillin or carboxy or ureido penicillin or ceftazidime in combination with an amino glycoside.

How can we report these results? We've added a comment to our pseudomonas report combination therapy with a beta lactam and amino glycoside should be considered, to further explain to the physician combination therapy is likely necessary for serious pseudomonas infections.

The next slide is Sanford guide recommendations for … primary agents, imepenem or meropenem, fairly broad spectrum agents prenolone and … alternatives being amsolbactam. Some strains of …are unique in light of the fact that they may be susceptible to amsolbactam and resistant to some other more common agents on your panel. So if you have a fairly resistant … have not explored the activity at the amsolbactam, this is something you might consider because in some cases it does represent a viable option if it tests susceptible.

That's not the case in the example on slide 65 where we're seeing a profile for … that is virtually resistant to all drugs on our panel. What are we

going to when see this type of scenario. On slide 66, I've consolidated those steps we might want to take when dealing with this type or organism. This type of phenomena can occur with phedomonus originosa and other non enter bactracia as well, much less common with the enter bactracia.

First, we need to consult with the physician before we extend additional resources in working up this organism. We want to find out if this islet is really important to patient management. If the physician believes this organism is likely colonizing the patient doesn't want therapy, there is no need arguing further testing. If the physician suggests this is an important organism and likely cause infection, you might want to suggest there be an infectious disease consult because there isn't a unique strategy for how to deal with these strategies. Each patient would be different and someone with extreme knowledge in treating the more problematic resistant organisms should be consulted on this case.

We may have other drugs available in our laboratory. For example, there may be some drugs on our panel and on our NCCLS tables that might be suggested for testing we haven't yet tested and don't have in our

laboratory. In those cases, we might want to get some supplemental drugs tested, and this is only if your physician is interested in those results.

Most recently, this CLSI had added break points for … polymixin. These were older agents used in the early 80s prior to the introduction of the third generation cephalosporins, and once those became available, it was thought those were the wonder, very broad spectrum drugs, and … polymixin, very toxic agents, were no longer necessary. Now that we've seen resistance emerge quick heavily to third generation cephalosporins, … polymixin drugs are being resurrected to treat some of these highly resistant bacteria that are not susceptible to any other current agent available.

On the bottom of 66, I've included a URL that might be helpful for some of you, particularly those that have cystic fibrosis patients and you're doing testing on their specimens, that this reflects the cystic fibrosis referral center at Columbia in New York. They actually work with the Cystic Fibrosis Foundation and have funding to do special susceptibility test on high resistant organisms from CF patients. They will do this free of charge. Sometimes they might also accept a fairly highly resistant pseudomonas … from other patients as well. So it's a valuable resource to

be aware of, and it's something you might want to share with your infectious disease physicians, particularly if they're dealing with CF patients.

Slide 7 talks more about the actual test calistin and polymyxin. These were just reintroduced for the first time in January of this year in the M100 S15. We have dis-difusion in MIC QC ranges for both calistin and polymyxin, but we only have MIC break for polymyxin B, but we have a comment that these can predict calistin MIC results, and the break points for polymyxin are listed on the side. If two or less is susceptible, four or greater is resistant. We can report polymyxin B MIC with a comment related to calistin. In terms of actually using a dis-diffusion methodology, the reason it hasn't been widely developed is because these drugs diffuse very poorly in auger, so it's difficult to get large enough zones to get precise measurements. But this is being explored further because it's conceivable that the distribution test may work for some species of gram negatives and not others. The other thing, too, is that we'll likely see MIC break points for calistin in addition to the polymyxin in our next version of our CLSI standards.

What about resistance among those organisms where these drugs would be of interest, for example, … pseudomonas, there is some resistance … calistin and polymyxin. It's rare to encounter polymyxin and calistin resistance among pseudomonas originosa. So in some institutions, calistin might be prescribed and the lab is not being asked to do testing because there is such a rare incidence of resistance to calistin among pseudomonas originosa.

Slide 68, let's show an example of how you can report results for this particular islet. I just used ours for the other drugs for simplicity, and here it did show the MIC for polymyxin since we don't have dis-diffusion method for polymyxin currently. Here, we can include the comment, what this means in terms of calistin, if we're probably MIC B susceptible, we could say … are calistin susceptible. If we're resistant, we could say the converse comment.

Slide 69, let's look at the drugs of choice recommended for … If you were to look at these and then at that recent addition to our CLSO table one and the listed suggested drugs to report, they're basically identical to those recommended in the Sanford guide to physicians for drugs to consider in treating infections caused by …

Next slide, there is an example of … from the blood that we might release from our lab at UCLA where we would do susceptibility tests on islets of … from sterile body sites. The drugs we'd report would be ceftazidime, minnow, imipenem… Now the reason we report imipenem, it's not at all effective against treating … on slide 69, it's not listed in the Sanford guide excerpt. But physicians think imipenem is going to get everything, so in our lab we automatically report imipenem as resistant on all … to remind physicians that this is not drug that should be considered for treating … infections. We also include a comment, when we've done the susceptibility test, if trimsulfa is not an option, infectious disease consult suggests it.

Slide 71, let's talk about the dilemma of infection versus colonization, particularly with some of these non enter bactracia. If we look at some of the literature related to this issue for … there was a very nice paper about a decade ago where they looked at it from a variety of sources among 59 patients. When they tried to document infection among them, they were only able to confirm 18 of these patients actually had a documented infections believed to be caused by the …

Similarly with …, there were a number of studies done looking at respiratory islets … when they looked at these islets, only 29% to 47% of the cultures that grew … actually represented patients infected with this organism. As you can see, these organisms were often colonizers.

How will we deal with this in the lab because we don't want to mislead physicians into treating organisms that are likely colonizers. In the next slide, there is just an example of a report for a gram stain and culture on a sputum specimen. The gram stain was very non-specific just showing oral flora and a few … normal oral flora. In this scenario, we're not suggesting to do susceptibility testing. This is likely colonization because you're not seeing organisms … white cells in the gram stain, only a few grew out on culture, and if we were to do susceptibility test on this organism, there is a great likelihood the physician would treat. We don't want the physician to treat islets that are likely to be colonizers and not causing the infection because it may over treat the patient, lead to problems with resistance and prevent the physician from looking further to see the true cause of the patient's problem.

Page 73, on this particular sputum culture, we see a few white cells and moderate gram negative rods on the gram stain. It grows out many as

needed … oral flora. By the way, this is not an NTCLS comment but something we're just doing at UCLA, we add the comments … are not routinely done because … is a colonizer when isolated from non-sterile body sites such as respiratory specimens, … is causing an infection, … test result should guide therapy and please contact laboratory, at that point we'd do testing.

Let's look at the example on 74 for …. a similar scenario. Here we modify our comment slightly saying susceptibilities for … are not routinely done because it's frequently a colonizer when isolated from non-sterile body sites such as respiratory. If it is causing infection, trimsulfa is the drug of choice. Here, too, this is another comment we're adding to emphasize to the physician to think twice about treating patients with these organisms because it may not necessarily mean that these patients are infected.

Slide 75, we'll summarize a little strategy for dealing with pseudomonas originosa and other non enter bactracia to make sure we test appropriate drugs on a routine panel, and we could use our table one and CLSI documents to guide us. For highly resistant strains, we need to determine of supplemental testing is necessary, and this will initially involve dialog

with the physician so as not to waste a lot of time and expend a lot of resources to provide information that is not going to be of any benefit for that patient's care.

Slide 76, I've listed the table from our CLSI documents that reflect verification of patient results. We have not discussed this table yet, we will in session four. One of the objectives for today was to make sure we mention about verifying or validating susceptibility results on gram negative organisms in terms of making sure they're as reliable as possible. This verification table is something that can help us in that regard, but I decided it would be best to discuss that under our quality assessment and lecture rather than do that now since we're out of time.

Move to slide 77, to conclude, our signature slide reflecting our master CDC Web site that has information on many aspects of susceptibility testing. For example, for some of the topics we discussed today, we have an ESBL case study on there. We have … case study and by the way, we also have case studies on our CD Rom that discusses a lot of the issues we've covered this afternoon.

With that, we might have a few minutes for questions.

R. Graham It looks like we do have some time. Thank you, Janet. If you have a question, now is the time.

Coordinator Our first question is from Wisconsin.

W This refers back to teleconference one. If I have a positive bacteria, rather it be … positive or negative and it's … oxacillin sensitive, is there any more screening I need to do for the MRSA?

J. Hindler You're talking about doing a oxacillin disk or MIC?

W MIC.

J. Hindler And a test resistant to oxacillin. Your question is, do you have to confirm oxacillin resistance by another method?

W Yes.

J. Hindler We're going to cover that next teleconference so hopefully you'll be there. But very briefly, there is not one hard and fast answer. It depends on your

confidence in the method you're using, if you've had problems before, the competency of your staff, the types of profiles you see in your organisms. But bottom line, I don't believe every laboratory use multiple methods to confirm MRSA these days.

Coordinator Next question is from Minnesota.

W Can we charge for ESBL testing?

J. Hindler I knew someone would ask that. Let me look that up and present it next time. Remember last time, we talked about that Ask It Web site? Did you check there and look under the archive section of compliance where there are so many answers to CPT coding questions there?

W I did not.

J. Hindler It might be there. I'll look it up, also, but see if you can find it. I believe you can, but I want to verify that.

Coordinator We have a question from Nebraska.

W We use the regular Vitech and if I'm understanding right, when we use the cards that have the ESBL on them, that's just the screen part. So if we get a positive ESBL on that Vitech card, we need to do confirmatory testing. Is that correct?

J. Hindler I'm glad you brought that up. The intent of that ESBL test on the Vitech card is that you do not have to do supplemental testing because that is the confirmatory test right there. Some labs do confirm it. Here, we do use the Vitech for urine enter bactracia, and our protocol, which is rather conservative because of our patient population, we're a … center so many of these ESBLs we're seeing are from very sick patients so we want to be sure we're absolutely correct. If it's ESBL positive, we're confirm it with the phenotypic confirmatory dis-diffusion test.

Also, if it's ESBL negative but there are other drugs on the panel that is might be suspicious for an ESBL producer, and we're only talking about e-coli and … then we'll do the ESBL confirmatory dis-diffusion test as well. But not everybody is doing that. Some labs that have the Vitech ESBL test are just accepting the Vitech ESBL results. I will tell you, there are some inconsistencies between the ESBL dis-diffusion confirmatory

test and the Vitech ESBL test on the card. So again, this is an individual decision each lab has to make.

I'm not saying there are lot of these problems. One reason there are some problems is because when that ESBL test was developed on the Vitech system, we didn't have 200 different types of ESBL enzymes around. It's really no fault of Vitech not being able to detect all of these. They did the best they could when they developed that test. They're in the process of modifying it, but the CLSI is thinking about modifying the whole approach to detecting ESBLs, so they're looking at all of these different ways to go with possibly reassessing the ESBL test.

W Thanks. Also, I spoke with Vitech about proteus and the ESBL cards and they say it does not test proteus so we have to find another way to do those.

J. Hindler I don't know if they've ever tried it, but I know it hasn't been FDA cleared for testing proteus. We're only talking about proteus from sterile body site islets that we're recommending you test right now. Do you have disk surround in your lab routinely?

W We do have disks but we don't have—

J. Hindler Well, a practical strategy might be if you have one suspicious, you could screen it. If you have some of the screening agents on your card and it screens suspicious for ESBL production, then a practical strategy may be to send it to a reference lab to get the confirmatory test done. Here, too, we're not talking about a large number of islets. I don't know about your place, but we don't see proteus … that often from sterile body site islets that would be suspicious for ESBL production. Would that be an appropriate strategy for you?

W Probably.

Coordinator Our next question is from Minnesota.

W From urine islets, I missed part of that and the rationale. Are you saying ESBL confirmatory should be done on urine islets or not?

J. Hindler If you know your patients have acute cystitis, you really don't need to do the ESBL testing because those organisms are confined to the bladder. You have so much drug in the bladder, it's going to overcome any ESBL

enzyme being produced by the bacteria so the drug will probably still be effective. However, most of us don't know if that urine specimen is from a patient that has acute cystitis or a more serious urinary tract infection such as kidney involvement. So I think it would be difficult to decide which ones you test and which you don't.

That little survey we sent out, there are some people that are doing ESBL testing on all sites except urine, and these are probably labs where most of their urines are coming from outpatients that are likely to have acute cystitis. In those cases, it certainly is essential to do ESBL testing on those organisms. I really think in the micro lab we need a way of getting more information on those urine samples. If we knew that was a patient with acute cystitis versus other types of infection, I think we can do a much better job of working up urine cultures and doing the appropriate susceptibility testing on these as well.

Coordinator At this time, there are no question.

J. Hindler I'd like to thank everybody for tuning in and for answering that question we sent out in terms of how many labs are doing ESBL testing. I hope those of you that answered no that are testing … particularly that has e-

coli … sterile body parts, I hope I've convinced you that it's really essential for either you to do the testing in your laboratory or send these organisms out to get the ESBL test done because we know if we don't do it on islets from blood and those patients organisms test susceptible to a third generation cephalosporin and the physician prescribes it, that failure can have a very adverse clinical outcome to include death, as you saw in Dr. Patterson's study. I hope that's one point you're able to take home.

If you need help developing a strategy in your particular lab, please e-mail me and we'll do whatever we can. I'm sure a lot of the problems some of you are having with these issues, other labs are having as well. We'll try to collate and post the questions we're getting towards the end of the series of teleconferences.

I look forward to talking to you in a couple weeks where we'll talk about gram positives and, one of our favorite subjects, detecting resistance in staph these days has becoming overwhelming. So if we think the ESBLs and MCs are difficult, there are more challenges with testing staph. Have a good two weeks, everybody. Thank you, Randy. Anything else to say?

R. Graham I'll provide this final reminder to all the participants to complete the online evaluation by March 24th. The directions should be in your handout. If you have any questions, talk to your site facilitator. Documenting your participation helps us to continue to bring high quality training programs in a variety of formats. That concludes our program of the National Laboratory Training Network. The other sponsors would like to thank Janet Hindler for this presentation. To reiterate what Janet said, part three is on March 31st. I hope you all will consider joining us for future programs and that you will make National Laboratory Training Network your choice for laboratory training. From the Minnesota Department of Health in Minneapolis, this is Randy Graham. Good day.