Washington State Department of Health

DOH 343-118 August 2014

Tuberculosis Training Series for LHJs and Community Partners

Transcript for Webinar #1: M. Tuberculosis Complex Sensitivities, 2014

Slide1: The title of the webinar is M. Tuberculosis Complex Sensitivities-2014; presented by WA State Public Health Laboratories, Tuberculosis Lab.

The presenter, Alla Ostash, is Microbiologist at Washington State Public Health Lab. Alla begins the presentation:

Hi this is Alla from the Washington State Public Health Laboratories and I will present a webinar on Mycobacterium tuberculosis complex sensitivity testing.

Slide 2: In our learning objectives we will cover the available sensitivity methods that our laboratory has. Also what are the challenges and limitations of drug sensitivity testing that we have to experience on pretty much a daily basis. And how to interpret the drug susceptibility testing results.

So what are the advantages and limitations of MTB sensitivity testing? Sensitivity first of all provides information on the sensitivity pattern of Mycobacterium Tuberculosis isolate. Based on this information the clinicians are able to adjust or modify the treatment regimen based on these results. But sensitivities require pure MTB isolates meaning this particular organism cannot be mixed with any other Mycobacterium organisms or other mycobacteria. It cannot be performed on clinical samples. It also takes about two to three weeks for results. And false resistance may also be detected and we will cover later in these slides why is that…

Slide 3: So what are the available sensitivity methods that we currently use? We have some that are FDA approved methods and these are primarily manufactured, available methods such as MGIT First Line DST and MGIT stands for Mycobacterium Growth Indicator Tube and a different method such as Versatrek and again they are FDA approved methods, and they are very automated so they are easier to use and a little bit less hands-on. There are also other methods that are available but not FDA approved. Those were primarily developed in the laboratories or some of the manufacturers are trying to come up with different methods for sensitivity testing. One of them is VersaTrak Sensititre which our lab is looking into current validation of it, molecular methods are available in various laboratories, ours is DRSS, which is drug resistance screening by sequencing, and also CDC has a method called MDDR, Molecular Detection of Drug Resistance and those were validated and implemented by laboratories in our state. And other laboratory development methods are available but we are not going to go into details in this webinar.

Slide 4: So MGIT sensitivities, how do they work? In order for the instrument to detect MTB growth it looks at the concentration of oxygen present in the MGIT tube. As the organism growth increases, less

and less oxygen is present in the tube. This particular method is able to provide growth of the organism within the 7-10 days. MGIT sensitivities provide the first line drug sensitivity testing such as Streptomycin, Isoniazid, Rifampin, Ethambutol and Pyrazinamide. Multiple laboratories using this method includes our laboratory, the Washington State Public Health Lab, also includes PAML Laboratory which is located in Sacred Heart Medical Center, Harborview performs the SIRE except for PZA and there are some various private labs that are using the same methods such as QUEST and LabCorp.

Slide 5: So here is the MGIT instrument. It is pretty much a big incubator that contains slots for the tubes. On the bottom of the drawer, as you can see, on the right side, the picture is open, there is laser located at the bottom of the drawer and it reads the fluorescence of the tubes every hour. So as the oxygen depletes in the tubes the fluorescence becomes brighter and brighter. And the instrument can detect the changes in fluorescence and call if the tube is positive.

Slide 6: So our laboratory performs these sensitivities on every first time positive MTB isolate from a patient. If the patient is still positive, culture positive, after three months from original collection date, you will run the sensitivities again. You need to make sure that there is no emerging resistance due to the interruption of treatment, for example or the patient is failing treatment all together. The MGIT sensitivities are set up at least once a week. SIRE requires about 10-14 days of incubation in order for the instrument to call it a valid run. And PZA may need to have up to 21 days to include some of the really slow growing organisms including M. bovis.

Slide 7: So what are our challenges while performing MGIT sensitivity testing? TB is a very slow-growing organism. Because of that it is a very poor competitor especially with the normal flora, and also other mycobacteria that are present in the clinical specimen may overgrow the culture. It is very hard to measure the growth concentration due to the fact that TB flocculates in the liquid culture and we will give you an example of flocculation in the next slide. That may be the main cause of over and under inoculation, which may ultimately cause in false results. MGIT sensitivities require extensive training for the personnel that are setting them up. It takes about 3-4 months of them training and up to 6 months before they become competent.

PZA drug is active in very low acidic environment. So it is very hard to achieve this particular condition in-vitro. And because of that MTB grows very poorly in PZA medium, so that is why it requires such a long incubation time. As you can see here in the picture on the bottom, the last two tubes appear a little different than the first three. The last two, especially the one before last is very highly contaminated with something other than TB. It is very milky and turbid and we would call this tube contamination and that would require us to double check that the isolate is pure and to repeat the sensitivity testing again.

Slide 8: So how TB growth in MGIT media is very important for us to distinguish if the culture is pure or is contaminated. Most of the time you see the TB growing just like in picture A, where you see the specs of growth that kind of, sort of appear as cottony clumps, and regardless how much you mix the culture, it will never turn into this very nice milky, even concentration throughout the tube, so when someone is setting up sensitivities it is very important that they are able to interpret how much organism they have before they set up so they don't over or under inoculate their sensitivity batches.

Slide 9: So here are some of the pictures of our sensitivity testing. As you can see in the top left picture we have INH resistant. You can see the little specs of growth that are appearing in the tube there. The PZA in here is actually PZA sensitive. You definitely see growth in the growth control because we require, to approve that the organism is actually able to grow in the media without the presence of any drugs. And the PZA tube appears to be clear which means no growth is detected.

Slide 10: So how do we report our sensitivity results? First of all we will provide you what method was used in order to perform sensitivities, we will give you the concentrations of the drugs that we tested with, we will give you the results, either sensitive, resistance, sometimes you will see unable to perform due to… the various reasons and we will provide you with the initials of the tech who run the test and the date the sensitivities were done. Also we will provide the comments that will include, which results are pending, some of the explanation of the results, like why we weren't able to perform a certain sensitivity testing as well as we will also communicate with you if the drug resistance be confirmed by the 7H10 plate method, which we will cover in a little bit.

Slide 11: So what are other sensitivity testing that are currently available on the market? So definitely there is a great development in the molecular methods in the last few years. Our laboratory offers the DRSS test which is Drug Resistance Screening by Sequencing where we detect mutations to four sites that can cause resistance to Rifampin, Isoniazid or Pyrazinamide. MDDR, molecular drug detection, I'm sorry, Molecular Detection of Drug Resistance that is offered by CDC and it is screening for a lot bigger panel of mutations that can rule out possibility of XDR as well as MDR.

Also there is commercial method and one of them we are considering to validate and implement in our laboratories such as VersaTrek Sensititre. It instead of determining if the sample is sensitive versus resistant, it tasks for a bigger sized concentration, which is called Minimal Inhibitory Concentration. And as I mentioned we are currently trying to validate it in our laboratory. This particular method can be completed within 10-14 days. It also contains some of the second line drugs in addition to the first line. And some of them are new to our laboratory that we were not testing before, such as: Cycloserine, Kanamycin, Moxifloxacin and Rifabutin. So we will provide a little wider panel of drugs available to physicians. Unfortunately it is unable to test for PZA due to the fact that PZA requires a really low acidic environment and this manufacturer was not able to be successful with their testing.

Slide 12: So this is how the VersaTrek, some of the images from the process. On the top left you will see the autoinoculator which is a very nice feature because you can inoculate a 96 volt plate that has various drug concentrations and various drugs already embedded into each well within 30 seconds or so. So the hands on is definitely going to be an improvement for our laboratory and we will be spending less time performing inoculation. On the right hand side you see a picture of various growth patterns for the organism. As you can see the arrow is pointing up and the increase of drug concentration so the very bottom row will have the smallest concentration. As we move vertically up, the drug concentration will be increased. The other two arrows, especially the one in the third row, is depicting Rifampin, which you can see due to the slight orange-ish tint of the well. And this particular strain would be resistant to Rifampin. Or it will have a really high minimal inhibitor concentration.

Slide 13: So in here we will go into the agar proportion method. Whenever we have any resistance to SIRE in our first-line drug sensitivity testing that is done in MGIT, we need to confirm it by agar proportion method or plate method as it is known to most of you people. And PZA is not available on plates, again, due to the low acidity; it will not, it cannot be performed on, on solid media. So PZA if you see any resistance, to it in the MGIT, in order to confirm it, it will be set up again on MGIT in order to make sure that our results are congruent. And the plates, how we make sure that the drug concentration is equivalent to whatever's needed to be done, we have purchased discs that are impregnated with known concentrations of drugs. And these discs are embedded into agar on our sensitivity plates. As the discs are being covered by the agar, the drug is able to diffuse throughout the agar providing the correct concentration. And in order for us to set up our cultures, our MTB isolates are diluted to acceptable concentration and inoculated onto sensitivity plates. And again, the training is here very important to make sure that the technicians are able to provide the proper concentration of TB positive cultures.

Slide 14: So here's our sensitivity plates. Once they are inoculated, they are incubated for three weeks to achieve sufficient growth and we will be reporting the sensitivity results after 3 weeks. On the left hand side you see a batch of sensi plates that are ready for inoculation. On the right hand side you can see the plates that contain sufficient MTB growth. I want to point out that each plate contains a control quadrant that has no drug added to it and we need to assure that this particular organism is able to grow without any drug. And then the plate for example on the bottom left, as you can see there is sufficient growth even with the drug present and that would be considered 100% resistant. And on the top right, there is a plate that you have a really good growth in the control quadrant but about 25 or so percent in the other quadrants with the drugs. So that would be considered 25 percent resistant. And then the last quadrant is considered sensitive. And the reason we report a 25-35 percent resistance is because if you have multiple concentrations tested, due to the lack of various drugs, the clinician may still want to use this drug that is resistant 25 percent with combinations of others because it may still be effective against, let's say 75 percent of the sensitive organisms.

Slide 15: So interpretations for the sensitivity plates, we usually set up two sets of dilutions, and they are inoculated to make sure that we have concordance of results and that one dilution is equal to other. And then QC strains must exhibit the expected resistance pattern, for example we have purchased the quality control strains and we know for sure that they will be one of them is resistant to Streptomycin and the other is resistant to Isoniazid. And these two strains must show us that the drug discs that we purchase actually work like they are supposed to be working. And the number of colonies that are present in the control quadrant and the number of colonies that are present in the drug quadrant are then estimated and reported and the percentage of drug resistance is then calculated and reported. For example, if you have 300 colonies in the control quadrant and about 150 colonies in the drug quadrant you would make the calculations to determine the percentage. And we would say that there would be 50 percent resistant to this particular drug.

Slide 16: So here are more pictures of our sensitivity plates. As you can see clearly the arrows are pointing to the drug discs, and this is on the picture on the left, and we can see that our Streptomycin is about 50 percent resistant to the concentration of 2.0 microgram/mL and sensitive to Streptomycin at

10 microgram/mL. And obviously sensitive to Rifampin due to the fact that there isn't any growth. We have nice growth and control quadrants because we need to make sure that the organism is able to grow in the absence of any drugs. And on the picture to the right we have a 100% resistance to INH at 0.2 microgram/mL and 1.0 microgram/mL so this is would something we report as 100 percent resistant to both concentrations of INH.

Slide 17: And how do we report the plate sensitivities? First of all, we will provide the name of the drugs that have been tested and also their concentrations. We will give you the results either or the interpretation of the results, either they are sensitive or resistant. We will give you the percentage of resistance, also provide the colony counts. Our growth control should always have a satisfactory growth counts on the report. Also the tech that performed the test as well as date and we'll give you the information, what dilutions we were using to set it up. So in this particular case it will be minus 3, minus 5 dilutions.

Slide 18: A little case study that we had recently, happened in our lab that, was depicting all these sensitivity testing, working together trying to give you as much information as possible. So we had performed a DRSS on the clinical sample after we ruled it out that it was TB with our NAAT testing. It was inconclusive for INH results due to the presence of peaks for both mutation and no mutation. So our molecular technicians were not able to give us satisfactory results with the inhA but we did not detect any mutations in our rpoB for Rifampin, katG for INH or pncA for PZA. When we set up the MGIT sensitivities result our inhA was resistant, so we were thinking, "Oh great, the reason it's resistant is because we actually have an inhA mutation". So we need to confirm this particular isolate with plate sensitivity method in order to make sure that that resistance is true.

Slide 19: So plates were set up and what we see here is INH sensitive, there is no growth on INH quadrant. But 100% resistance to Ethionamide, so what is going on here? Most likely, in our conclusion, we determined that we had a mixed population of TB, it is the same strain but the mutation in inhA region is only found in some organisms and not all. So in this particular case we determined that there may be an emerging resistance to INH. Also some mutations in the inhA region can overexpress the inhA protein which mediates co-resistance to INH and Ethionamide. So that is why we see the resistance to Ethionamide on our plate method. This was all able to be estimated due to the fact that we completed all these various testing that we did and rule out the INH resistance as well as Ethionamide resistance.

Slide 20: Thank you very much for attending this webinar and also would like to thank the TB Laboratory folks for providing their time and help with presenting this. And to the TB Program staff for organizing all our sessions.

Slide 21: Here is the contact information for myself and our section supervisor, Craig Colombel, if you have any questions, feel free to either call or email us. And we will open now for questions.

Slide 22: (Shows a picture of a human figure leaning up against a question mark. There were no questions so webinar ends).