Welcome to the Association of Public Health Laboratories, Essentials for the Mycobacteriology Laboratory, Promoting Quality Practices. This presentation is molecular detection of drug resistance.
1.2 Introduction

Notes:

This presentation is Molecular Detection of Drug Resistance. So let's start with an introduction, and talk about why the increased interest in molecular diagnostics for TB.
1.3 Why the Increased Interest in

**Why the Increased Interest in Molecular Diagnostics for TB?**

- Goal of TB elimination and recognized need for more rapid diagnosis
- Updated CDC guidelines for use of NAAT in the diagnosis of TB (June 2009)
- Advances in technology allowing the ability to simultaneously detect *M. tuberculosis* complex (MTBC) and mutations associated with drug resistance
- FDA market authorization of GeneXpert MTB/RIF assay (2013)
- Decreasing price and increased access to platforms/reagents for laboratory developed tests (LDT)

**Notes:**

In United States we're trying to reach elimination of tuberculosis. And with this goal, we need to have more rapid diagnosis, so the patients are diagnosed and treated before they spread the infection to others. Secondly we have updated CDC guidelines from 2009 which established the use of nucleic acid amplification testing or NAAT. It's the standard of practice for diagnosis of tuberculosis for each patient with signs and symptoms of TB. Then there are advances in technology, which allow simultaneous detection of TB complex and mutations associated with drug resistance to quickly identify patients who have drug resistant disease. The US food and drug administration or FDA, gave market authorization for GeneXpert, MTB/RIF, this is a commercial product, in 2013. We also have decreasing price, and increased access to DNA sequencing platforms and reagents for laboratory developed tests or LDTs.
1.4 Why the Increased Interest in

Why the Increased Interest in Molecular Diagnostics for TB? (2)

- Shift in laboratory workforce and loss of expertise for conventional TB testing methodologies
- Molecular diagnostics are commonplace for other diseases; can capitalize on skill sets of existing molecular workforce and infrastructure
- Global roll-out of GeneXpert MTB/RIF assay
- WHO policy for line probe assays and GeneXpert MTB/RIF
- Incorporation of molecular diagnostics in drug resistance surveys around the world

Notes:

So second slide on this topic, about increased interest and molecular diagnostics for TB, is that we have a shift in the laboratory work force. We have people coming out of college with a good education in molecular biology and less in conventional TB testing methodologies.

Molecular diagnostics are common place for other diseases. So for example, with Cepheid GeneXpert, a laboratory may have this instrument to use with other infectious diseases as well. Then there is the global roll out of GeneXpert MTB/RIF assay. It's widely used in developing countries. And there's a great body of literature on application of this method and its performance characteristics. The World Health Organization has a policy statement that promotes the use of line probe assays as well as the GeneXpert, MTB/RIF. And molecular diagnostics are being increasingly used worldwide in drug resistant surveys.
1.5 Advantages of Molecular Detection of Drug Resistance

- Phenotypic methods are not timely due to slow growth of MTBC
  - Turnaround times can be extended, requiring weeks to months from specimen receipt
- Molecular assays can reduce turnaround time to hours to days
- Can provide information on drug resistance in cases where phenotypic results are unavailable (e.g., no growth, contamination, or mixed with NTM)

Notes:
So what are the advantages of molecular detection of drug resistance? The phenotypic or cultural based methods are not timely because of slow growth of mycobacterium tuberculosis complex. Turnaround times for cultural based methods can be extended, requiring weeks to months from the time the specimen is received in the laboratory. I can still remember instances where in the United States we had patients in prison dying of tuberculosis, which was drug resistant when they had HIV, before the laboratory cultural based result came back.

So with the molecular methods, we can diagnose drug resistant TB in a timely manner and get people with drug resistance treated properly with a proper regimen much more quickly. So with the molecular assays we can reduce the turnaround time from weeks to months to hours to days instead. Which is much more satisfactory for prompt initiation of the right treatment. Molecular methods also can provide information on drug resistance in cases where the cultural based or phenotypical results are unavailable. For example if you got a mixed culture of TB bacteria, with non tuberculosis micro bacteria. Or with non-micro bacteria contaminates. You can still perform the molecular techniques and get a drug susceptibility result as well as identification of the TB.
A second slide of advantages on molecular detection of drug resistance describes that phenotypic methods, or cultural based methods, are known to be an imperfect gold standard. In some cases the molecular methods can provide more accurate results. For example, mutations resulting in low level but clinically significant rifampin resistance. And we're going to talk more about this later.

And then there's the fact that some drugs can be very difficult to test phenotypically. Ethambutol for example gives us a lot of headaches. On Agar proportion drug susceptibility testing, with an ethambutol drug, micro colonies can form, and we really don't know how to interpret these. And then also with ethambutol, and the more rapid broth-based methods, like MGIT, or micro bacterial growth indicator tube, it's been reported that we get false susceptibility sometimes.

And then there's pyrazinamide. Pyrazinamide is a drug which is active in an acidic
environment. And the acid medium in which we do the pyrazinamide susceptibility testing is not ideal for growing TB bacteria. So this creates problems. And one of the problems is that if you heavily inoculate the pyrazinamide drug susceptibility test, the inoculum can increase the pH, can bring it more toward neutrality, and result in false resistance because the drug is not active at a neutral pH. And another factor is that for second line drugs, phenotypic methods are not well standardized.
Considerations for Use of Molecular Assays

- Molecular assays do not yet replace phenotypic drug susceptibility testing (DST)
- Not all laboratories have infrastructure or need to perform testing in-house
- High complexity assays requiring subject matter expertise
- With the exception of GeneXpert MTB/RIF, molecular assays for the detection of drug resistance are LDT or research use only (RUO)
- Positive predictive value decreases in populations with low prevalence of resistance
- Referral for molecular detection of drug resistance is available for specimens or isolates

Notes:

So let's talk about some considerations that you may want to think about if you want to make a decision about whether to perform molecular assays in your in your own laboratory. Keep in mind that molecular assays do not yet replace the culture based or phenotypic drug susceptibility methods. And that not all laboratories have the infrastructure or the need to perform the susceptibility testing by molecular methods in house. These are high complexity assays that require subject matter expertise.

Now the exception of the GeneXpert MTB/RIF, the molecular assays for detecting drug resistance, are laboratory developed tests or research use only tests, which require extensive validation. Another factor is that the positive predictive value of detecting drug resistance by a molecular method decreases in populations that have low prevalence of resistance. And remember that referral for molecule detection of drug resistance is available for specimens or isolates.
1.8 Mutations and Drug Resistance

Note:
Okay let's talk about mutations and drug resistance. Not all mechanisms of resistances are known. So if we look for a mutation, and we don't find it, this does not always mean that the organism is going to be susceptible. Resistance could be due to other mutation that we don't know about. And not all mutations are associated with phenotypic drug resistance. Some mutations are silent or synonymous. There's no change in the amino acid sequence of the protein that's being coded for. The mutation also may not cause a significant change in the protein expression or structure or function, so that drug resistance does not result from the mutation.
1.9 Mutations and Drug Resistance (2)

Notes:

Association of a mutation with drug resistance requires data related to its correlation with phenotypic drug resistance, functional genomics, and clinical outcomes. These are difficult to develop data regarding, and so it's going to take some time for us to develop the information that we need to associate particular mutations with drug resistance. Association with clinical outcomes is difficult due to multidrug therapy which is always used to treat TB disease.
1.10 Intrinsic Drug Resistance

Intrinsic Drug Resistance

- Hydrophobic cell envelope (permeability barrier)
- Drug efflux systems which can pump toxic substances out of cell
- Drug modifying enzymes can alter the drug configuration
- Intrinsic PZA resistance seen with some members of the MTBC due to lack of pyrazinamidase activity
  - *M. canettii, M. bovis* and *M. bovis* BCG (other members of MTBC are usually susceptible)

Notes:

We have intrinsic drug resistance of TB bacteria. One of the reasons is that they have a hydrophobic cell envelop, which can make it difficult for drugs to permeate into the organism. There are drug efflux systems, which can pump toxic substances like anti TB drugs out of the cell. I think this is particularly important in large TB lesions or cavitary lesions in the lung. Before the therapy has really begun to have an effect, there can be very large lesions. The drug concentrations in these large lesions may be low, and so the drug efflux systems, which pump drugs out of the bacteria can reduce the effect of the drug treatment.

Then there are drug modifying enzymes that can alter the drug configuration. For example aminoglycoside modifying enzymes can alter the structure of Amikacin or Clarimyosin and reduce their effect. And lastly we have intrinsic PZA resistance, which is seen with some members of the TB complex because they lack pyrazinamidase activity. And these include *mycobacterium canetti, M. bovis*, and the bovis BCG strain. But other members of the TB complex are usually susceptible to Pyrazinamide.
1.11 Development of Drug Resistance

**Notes:**

So how does development of drug resistance occur? Spontaneous mutations occur in the DNA of all cells, and the mutations can change the structure of a protein, which is a drug target. So that the protein still functions but it’s no longer inactivated by the drug, and then TB can grow in the presence of the drug. Resistance is linked to large bacterial populations, because mutants resistant to any drug naturally occur on an average once in every $10^8$ cells. So mutations for isoniazid resistance may occur more frequently. With pulmonary tuberculosis, the cavities contain $10^7 - 10^9$ organisms. By using two antibiotics, the chances for both targets to be mutated and resistant to both drugs is extremely small ($10^8 \times 10^8 = 10^{-16}$). This is the rationale for treatment regimens with more than one drug.
### 1.12 Other Factors Influencing the Development of Drug Resistance

#### Other Factors Influencing the Development of Drug Resistance

- **Metabolism of bacilli shifted to dormancy**
  - Impaired/ decreased drug uptake by *M. tuberculosis* cell
- **Penetration of drugs to various body sites**
  - Suboptimal drug concentration at some body sites
    - Less than therapeutic concentration
- **Impaired drug absorption due to underlying host conditions such as HIV/AIDS, diabetes**
- **Treatment with inappropriate drugs, combinations or dosages**
- **Interrupted or irregular treatments**
- **Incomplete treatments**
  - Limited duration (e.g., stop early because feels good)
  - Required number of doses not taken

### Notes:

There are some other factors that influence development of drug resistance, and one is that the metabolism of TB bacilli sometimes is shifted in cavitary legions to a dormant state. And when there's dormancy, there's impaired or decreased drug uptake by the TB cells.

Another issue is penetration of drugs to various body sites. You may have suboptimal drug concentrations at some body sites like large cavitary legions. And then the concentration at these sites may be less than the therapeutic concentration that's required for affective treatment.

Another thing that happens is impaired drug absorption due to underlying host conditions such as HIV/AIDS, or diabetes, and also malnutrition. What we want to avoid is treatment with inappropriate drugs, or combinations, or dosages.

Sometimes there are interrupted or irregular treatments caused by failure of the patient to continually take the medication. And there can be incomplete treatments because of limited duration. Patient can stop early because they feel good or because they have a side effect and then they don't take the required number of doses to effectively treat the disease.
1.13 Primary vs. Acquired Drug Resistance

### Notes:

So we have primary and acquired drug resistance. With primary drug resistance, the patient's TB bacilli are resistant at the start of treatment, and this is true even though the patient was never treated in the past, implying that there was transmission of drug-resistant bacilli.

Then there can be acquired drug resistance, which is a case where the patient had drug susceptible bacteria at the start of treatment, which became drug resistant during treatment. And this is what we want to avoid. We want to avoid medical practices that lead to development of acquired resistance.
So let's talk now about anti TB drugs and their associated genetic targets. And look first at the TB primary drugs and their mechanism of action.
2.2 TB Primary Drugs and Mechanisms of Action

Notes:

Some of the drugs, isoniazid and ethambutol, on the upper left here, you'll notice they inhibit cell wall synthesis. Isoniazid by inhibiting mycolic acid synthesis, and ethambutol by inhibiting a arabinogalactan synthesis. Pyrazinamide targets are a little less clear, it probably disrupts the plasma membrane and also effects energy metabolism or ATP production. And you'll notice on the upper right here, that rifampin it's well-known to inhibit RNA synthesis.
2.3 Rifampin

Rifampin

- Rifampin (RIF) is the most important drug in treatment of TB
- Targets the β-subunit of the RNA polymerase to inhibit the initiation of transcription
- RIF resistance (RIF-R) can be a marker for MDR TB as mono-rifampin resistance is not common
- rpoB most common gene involved in RIF-R
  - ~96% of RIF-R strains carry mutations in the 81 base pair rifampin resistance determining region (RRDR)
  - Secondary site for resistance at codon 176

Notes:

So going to the next slide, rifampin is the most important drug in treatment of TB. It targets the beta subunit of the RNA polymerase to inhibit transcription. And rifampin resistance can be a marker for MDR TB because mono-rifampin resistance is not common. You may recall, I mentioned earlier that resistance to isoniazid occurs more frequently than resistance to other drugs. This is why INH resistance usually develops first. And when you find rifampin resistance, you can usually presume that it is isoniazid resistant as well. The RNA polymerase gene or rpoB is the most common gene involved in rifampin resistance. About 96 percent of rifampin resistance strains carry a mutation in the 81 base pair, rifampin resistance determining region or RRDR. Some have a mutation instead at codon 176, which is outside the RRDR.
Notes:

So let's talk about some of the common mutations that occur in the rifampin resistance determining region or RRDR. And this slide had some data from a 1998 study by Ramaswamy and Musser. And it shows in their study what were the frequency of mutations in the RRDR associated with rifampin resistance. If we look toward the right, we'll notice that at codon 531, 41 percent of rifampin resistance was due to mutation at this codon. There is other hot spots as well. At codon 526, this is 36 percent of rifampin resistance is due to a mutation here. And a little further to the left of codon 516, 9 percent of rifampin resistance in this particular study were due to mutations at codon 516. The numbers may differ slightly between one study and another.
2.5 Considerations for Detecting RIF-R

Considerations for Detecting RIF-R

- Some mutations are associated with low-level but presumably clinically significant RIF-R that may be missed by commercial broth systems
  - Examples: Leu511Pro, Asp516Tyr, His526Asn, His526Leu, Leu533Pro, and Ile572Phe (outside the RRDR)
  - May result in discordance between phenotypic and molecular methods
  - Concern that missing RIF-R could result in poor clinical outcomes

- Some mutations that are associated with low-level RIF-R may retain rifabutin susceptibility
  - Examples: Asp516Val and His525Leu

Notes:

Now let's move on to some considerations for detecting rifampin resistance. There are some mutations that are associated with low level, but presumably clinically significant, rifampin resistance that may be missed by a commercial broth systems such as micro bacteria growth indicator tube. So there are some examples of mutations at 511, and 516, and 526 codons. As well as 533 and 572. And 572 as I mentioned earlier, that's outside of the rifampin resistance determining region. So these mutations may result in discordance between the culture based or phenotypic testing and the molecular methods. So we have a concern that if we are using cultural based methods, we can be missing some rifampin resistance, and that can lead to poor clinical outcomes. Note that there are some mutations that are associated with low level rifampin resistance that retain rifabutin susceptibility, and we have an example of a couple mutations at codons, 516 and 525, that have this phenotype. They are resistant to rifampin but they are susceptible to rifabutin.
2.6 Isoniazid

Now let’s talk about isoniazid. This is commonly used to treat contacts with latent Tuberculosis infection. It’s a prodrug that must be activated by catalyzed peroxidase and coated by the katG gene. And it affects mycolic acid biosynthesis. inhA and katG are the most common genes associated with INH resistance (INH-R).

- Most common mutations associated with high-level INH-R are found in katG
  - Ser315Thr and Ser315Asn
- Most common mutations associated with low-level INH-R are found in the inhA promoter region
  - C-15T, T-8C

Notes:

Now let’s talk about isoniazid. This is commonly used to treat contacts with latent Tuberculosis infection. It’s a prodrug that must be activated by catalyzed peroxidase and coated by the katG gene. And it affects mycolic acid biosynthesis. inhA and katG, are the most common genes associated with INH resistance. And the most common mutations associated with high level INH resistance are found in katG. And these are at codon 315. The most common mutations associated with low level INH resistance are found in the inhA promoter region. And there are two sites that are mentioned here.
2.7 Considerations for Detecting INH Resistance

<table>
<thead>
<tr>
<th>Considerations for Detecting INH Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Mutations in \textit{inhA} promoter region are associated with cross-resistance to ethionamide</td>
</tr>
<tr>
<td>• (\sim15%) of INH-R strains do not have an \textit{inhA} or \textit{katG} mutation</td>
</tr>
<tr>
<td>• Analysis of additional loci may improve detection of INH-R</td>
</tr>
<tr>
<td>• \textit{fabG1(mabA)}</td>
</tr>
<tr>
<td>• \textit{ahpC}</td>
</tr>
<tr>
<td>• \textit{kasA}</td>
</tr>
<tr>
<td>• \textit{ndh}</td>
</tr>
<tr>
<td>• \textit{furA}</td>
</tr>
<tr>
<td>• \textit{mshA}</td>
</tr>
</tbody>
</table>

Notes:

So let's talk about some considerations for detecting INH resistance. Mutations in the \textit{inhA} promoter region are associated with cross-resistance to ethionamide. About 15 percent of INH resistance strains do not have an \textit{inhA} or \textit{katG} mutation. It can be useful to look at additional loci to improve the sensitivity of detecting INH resistance. Most notably if we can look for mutations in the \textit{fabG1}, or \textit{mabA} gene, or \textit{ahpC}. Mutations in these genes have been associated with INH resistance. As well as the other four that are listed here. \textit{kasA}, \textit{ndh}, \textit{furA}, and \textit{mshA}.
Pyrazinamide (PZA) has important sterilization activity that shortens the duration of treatment when used in combination with RIF.

A prodrug that is converted to its active form, pyrazinoic acid, by a pyrazinamidase encoded by \textit{pncA}.

Inactivation of pyrazinamidase/nicotinamidase due to \textit{pncA} mutations is a major cause of PZA resistance (PZA-R).

Notes:

Pyrazinamide, or PZA, has an important role in sterilization activity, and it can shorten the duration of treatment when it's used in combination with rifampin. Before we had PZA, TB treatment regiments lasted for a year. Now incorporating PZA in the regiments enables them to be shorted for drugs susceptible TB to six or nine months. PZA is a prodrug that's converted to its active form, pyrazinoic acid by pyrazinamidase, an enzyme coated for the \textit{pncA} or, "pink A" gene. An activation of pyrazinamidase/nicotinamidase due to \textit{pncA} mutations is a major cause of PZA resistance.
2.9 Considerations for Detecting PZA-R

Considerations for Detecting PZA-R

- 70-90% of PZA-R isolates have a *pncA* mutation
  - Detection of mutations within *pncA* may be more reliable than current phenotypic testing
- *pncA* consists of 187 codons
  (186 amino acids + stop codon)
- No “hotspot” for mutations; genetic variations throughout
- *M. bovis* and *M. bovis* BCG are naturally resistant to PZA due to a His57Asp substitution within *pncA*

Notes:

Let's talk about some considerations for detecting PZA resistance. 70 to 90 percent of PZA resistant islets have a *pncA* mutation. And detection of mutations with a *pncA* may be more reliable than current phenotypic testing. If you read studies that compare the accuracy of DNA sequencing of the *pncA* gene to a cultural based susceptibility testing for PZA, please remember that the cultural based testing is an imperfect standard, and that sometimes the sequencing may be right rather than the reference cultural based method. The *pncA* gene consists of a 187 codons, a 186 amino acids, plus a stop codon. There's no hot spot for the mutations. Genetic variations can occur throughout the *pncA* gene. *Mycobacteria bovis* and bovis BCG are naturally resistant PZA due to a mutation at codon 57, His57Asp substitution within *pncA*.
2.10 Ethambutol

Ethambutol (EMB) inhibits the *embABC* encoded arabinosyl transferases required for formation of a major cell wall component.

- *embB* most common gene associated with EMB resistance (EMB-R)
  - 50-60% of EMB-R isolates have mutations at codon 306
    - Met306 → Val, Leu, Ile
  - Other mutations
    - Asp354Tyr
    - His406 → Ser, Asp
    - Met423Ile

Notes:

Okay let's move on to ethambutol. This is a drug that causes a lot of headaches for TB laboratories doing drug susceptibility testing. This drug inhibits the arabinosyl transferases, which are coated for by *embABC* genes. And these arabinosyl transferases are required for formation of a major cell wall component. Mutations in the *embB* gene are the most common ones associated with ethambutol resistance. 50 to 60 percent of ethambutol resistance strains have mutations at codon 306. In the *embB* gene. There are other mutations in *embB*. For example at codons 354, 406, and 423.
2.11 Considerations for Detecting EMB-R

Considerations for Detecting EMB-R

- Silent (e.g., Leu355Leu) and neutral (e.g., Glu378Ala) mutations that do not confer drug resistance have been detected
- Mutations in *embA*, *embB*, and *embC* reported in clinical isolates of *M. tuberculosis* resistant to EMB
- Variability in minimal inhibitory concentration (MICs) for mutations at codon 306 and 406

Notes:

So some considerations now for our detecting, ethambutol resistance. Some silent and neutral mutations that do not confer drug resistance have been detected. Mutations in *embA*, *embB*, and *embC* are reported in clinical isolates of *M. tuberculosis* that are resistant to ethambutol. There is some variability in the minimal inhibitory concentrations, or MICs, when you have mutations at codons 306 and 406. And we have found that not every strain with the same *embB* mutation will have the same MIC. There's a range of MICs associated with each particular mutation.
2.12 Fluoroquinolones

Fluoroquinolones

- Common drugs: moxifloxacin (MOX), levofloxacin (LVX), ofloxacin (OFL), ciprofloxacin (CIP)
- Most effective second-line drugs for patients with MDR TB
- Inhibit DNA gyrase that is essential for DNA replication, transcription, and recombination
- $gyrA$ is the most common gene involved in FQ resistance (FQ-R)
  - $gyrA$ mutations in the quinolone resistance determining region (QRDR)
- $gyrB$ mutations also associated with resistance

Notes:
Let's talk next about fluoroquinolone. These are very important drugs for treating multidrug resistant TB. If you have MDR TB, which is resistant to isoniazid and rifampin, you'll very likely be treated with a fluoroquinolone. And the commonly used drugs are moxifloxacin, levofloxacin, and ofloxacin. Ciprofloxacin, while it's a fluoroquinolone, is no longer commonly used for treating tuberculosis. These are the most effective second line drugs for patients with MDR TB. They inhibit the DNA gyrase, which is essential for DNA replication and transcription and recombination. And $gyrA$ is the most common gene involved in fluoroquinolone resistance. For $gyrA$ the mutations most often occur in what's called the quinolone resistance determining region or QRDR. But $gyrB$ mutations are occasionally also associated with resistance.
Notes:

So let's take a look at the next table and the next slide. Common gyrA and gyrB mutations. As I mentioned the gyrA mutations are much more commonly associated with resistance than the gyrB mutations. And the codons involved are 88, 90, 91, and 94. Patterns of cross resistance may vary.
2.14 Considerations for FQ-R

Considerations for FQ-R

- Effect of *gyrA* mutation combinations
  - Thr80Ala: Susceptible
  - Ala90Gly: Resistant
  - Thr80Ala + Ala90Gly: Susceptible
- Limit of detection and variability in FQ DST results
  - Some FQ-R populations may not be detected using molecular methods
- Patterns of cross-resistance vary among mutations
- Clinical relevance not well understood
- Some laboratories may reflex to MOX MICs when certain *gyrA* mutations are observed
- Some *gyrB* mutations may be associated with low-level MOX-R (but not consistently)

Notes:

Well let's talk about some considerations for fluoroquinolone resistance. And talk first about the effect *gyrA* mutation combinations. It's been discovered that you can have a mutation in the codon 80 of *gyrA*, which is associated with susceptibility to fluoroquinolones.

On the other hand the mutation, at codon 90 of [allanine to glycine] is associated with resistance. But if both mutations are present, at codons 80 and 90, the organism is susceptible. So this is an unusual combination of two mutations with a sort of anomolous susceptibility pattern. There is a limit of detection and some variability and fluoroquinolone drug susceptibility testing results. If you have a mixture of susceptible and resistant TB bacilli, these may not be detected by the molecular technique.

On the other hand, if there's a small percentage of the population which is resistant to fluoroquinolones this may still be detected by the cultural based methods. Which is probably a good reason to do both methods, both the molecular and cultural
based testing for fluoroquinolone resistance.

The patterns of cross resistance vary among the mutations and moxifloxacin, the newest of the fluoroquinolones, may still be effective when there are gyrA mutations. Some laboratories may reflex to moxifloxacin MICs when certain gyrA mutations are observed to find out what is the quantitative level of susceptibility or resistance to the newer fluoroquinolone. Some gyrB mutations may be associated with low level moxifloxacin resistance but not consistently.
2.15 Second-line Injectables

Notes:

Let's talk now about second line injectable drugs, which include amikacin, kanamycin, and capreomycin. The most common genes involved in resistance are mutations in *rrs*, which codes for 16S ribosomal RNA. The *eis* gene, which codes for aminoglycoside acetyltransferase. And the *tlyA*, which codes for methyltransferase that modifies the 16S and 23S ribosomal RNA. So some considerations for detecting resistance, some other mutations that we don't know about may cause resistance, and there's inconsistent, cross resistance patterns, which we will talk about in the next slide. And there could be variable critical considerations for capreomycin.
## 2.16 Common rrs, eis and tlyA Mutations

### Common rrs, eis and tlyA Mutations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>rrs</td>
<td>A1401G</td>
<td>Variable cross-resistance patterns reported (KAN, AMK, and CAP)</td>
</tr>
<tr>
<td></td>
<td>C1402T</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G1484T</td>
<td></td>
</tr>
<tr>
<td>eis</td>
<td>C-14T</td>
<td>KAN</td>
</tr>
<tr>
<td></td>
<td>G-10A</td>
<td>KAN</td>
</tr>
<tr>
<td></td>
<td>G-37T</td>
<td>KAN</td>
</tr>
<tr>
<td>tlyA</td>
<td>C insertion in 218L</td>
<td>CAP</td>
</tr>
<tr>
<td></td>
<td>Asn236Lys</td>
<td>CAP</td>
</tr>
<tr>
<td></td>
<td>Leu150Pro</td>
<td>CAP</td>
</tr>
<tr>
<td></td>
<td>ΔAla codon 23</td>
<td>CAP</td>
</tr>
</tbody>
</table>

### Notes:

Okay let's talk about the slide now about common rrs, eis, and tlyA mutations. You'll notice that mutations in the rrs gene can cause resistance to all three drugs, kanamycin, amikacin, and capreomycin. Although there's variable cross resistance. eis mutations on the other hand, are only associated with resistance to kanamycin. Strains with these mutations in eis will still be susceptible to the amikacin or the capreomycin. And the tlyA, or the t-ly-A, mutations are associated with resistance to capreomycin only, and not the other injectable drugs.
### 2.17 Most Common Loci with Mutations Conferring Resistance

<table>
<thead>
<tr>
<th>Drug</th>
<th>Common Gene/Loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampin</td>
<td>rpoB</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>inhA, katG</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>pncA</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>embB</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>gyrA, gyrB</td>
</tr>
<tr>
<td>Second-line Injectables</td>
<td>eis, rrs, tlyA</td>
</tr>
</tbody>
</table>

**Notes:**

Alright sort of the summary slide is the most common loci associated with mutations conferring resistance. Now you notice that for rifampin, the most common gene causing resistance are *rpoB*, for isoniazaid, *inhA* and *katG*. For the pyrazinamide, the *pncA*. Ethambutol, *embB*. For fluoroquinolones, its mutations in the *gyrase* genes, *gyrA*, and *gyrB*. And then for the second line, or injectable drugs, *eis*, *rrs*, and *tlyA* mutations are associated with resistance to these.
2.18 Caveats for Results Interpretation

Caveats for Results Interpretation

- Finding a mutation does not always result in phenotypic resistance
- A wild type sequence does not confirm a strain is susceptible
- We do not know every possible locus leading to resistance for any antibiotic

Notes:

Now let's review some caveats for results interpretation. Finding a mutation does not always result in phenotypic resistance. And a wild type sequence in the gene sequences that we look at, do not confirm that a strain is susceptible. Because we do not know every possible locus leading to resistance for any antibiotic.
3. New Anti-TB Drugs

Notes:

Okay let's talk about new anti TB drugs.
3.2 Bedaquiline (Sirturo™)

**Notes:**

For many years there were no new anti TB medications. But now we have bedaquiline, or Sirturo, which was approved in 2012 by the US Food and Drug Administration for use in combination therapy, to treat adults with pulmonary multidrug resistant TB, when there's no other alternatives. CDC is asking that when a patient is being treated with bedaquiline, that monthly follow up cultures be collected from patients and tested for resistance. We know that mutations generated the *atpE* gene, lead to bedaquiline resistance in vitro, but these have not yet been identified clinically.

Interestingly, clofazamine resistance strains of TB have shown cross-resistance to bedaquiline as a result of up-regulation of an efflux pump. Currently resistance to bedaquiline is determined using MIC testing, and increases in MIC have been seen in patients, who fail to convert, or relapse, following bedaquiline treatment. We need to work to develop standards for bedaquiline susceptibility and by cultural based methods.
3.3 Other New Drugs

There are some other new drugs including delamanid, and this has been granted marketing authorization in 2014 in Europe for use in MDR TB. It’s a nitro-dihydroimidazooxlazole. It inhibits mycobacterial cell wall synthesis. Unfortunately it cannot be given together with bedaquiline because both of these drugs can contribute to cardiac arrhythmias, and so there is a concern that if both are given that arrhythmias will develop. We also have PA-824. This is a drug which has been around for a while but now finally is an undergoing clinical trials. It's a bicyclic nitroimidazole which is active against both replicating and latent MTB. And resistance to PA-824 is commonly mediated by loss of a specific glucose-6-phosphate dehydrogenase (FGD1) or its deazaflavin cofactor F420. MIC testing and mechanisms of resistance are being evaluated.
4. Sequence-Based Molecular Methods

Notes:

We'll talk now about sequence-based molecular methods for detecting drug resistance. These methods have added a great deal of complexity to our understanding and a lot more depth to our understanding of drug susceptibility testing.
4.2 Considerations for Sanger Sequencing

We'll talk first about Sanger sequencing. Now Sanger sequencing can be used either directly with patient's specimens or isolates, as you see at the bottom of this slide.

Sanger sequencing has the advantage that it can sequence long DNA segments. It's easy to customize, so you can take a look at the gene region that you want, and it can detect mixed populations of susceptible and resistant bacilli, provided that neither population falls below 30 percent. It can let you find numerations and gives you the actual DNA sequence, which is more informative in some cases then the susceptible or resistant result that you get in cultural based testing.

Disadvantages include that its labor intensive. The equipment is expensive as well as the reagents, and there is a limit to the throughput that you can have when you're doing Sanger sequencing.
4.3 Sanger Sequencing Results

Notes:

Here's an example of Sanger sequencing results. You can compare the results that you get with the strain that you're sequencing to a known reference standard.
4.4 Considerations for Pyrosequencing

Let's talk next about pyrosequencing. This can be used for sequencing DNA from specimens or from isolates that are grown in culture. It has a major advantage in that it's rapid. And the major disadvantage is that pyrosequencing is only accurate for short sequence reads.

As with Sanger sequencing, you can detect specific mutations. It's transparent with the pyrogram to see the results and you can be clear about the quality of the sequence data. It's easy to customize and that you can look at specific region that you choose. And with pyrosequencing, you can have high throughput, you can test large numbers of samples at the same time.

It is labor intensive. And there's a problems with pyrosequencing. One of the disadvantages that you can have homopolymer stretches. That is more than three of the nuclear tides can sometime lead to incorrect interpretations. This is not practically turned out to be a serious problem with the DNA sequences that we look at, when we're detecting drug resistance. New mutations detected by pyrosequencing may need to be confirmed by Sanger sequencing to make sure there's not homopolymer error. It can be more difficult to detect mixed populations with pyrosequencing then it is with Sanger sequencing.
4.5 Pyrosequencing Workflow

Notes:

Okay let’s take a look at pyrosequencing work flow. You can see that the steps: PCR about two hours, and then 45 minutes for single strained DNA preparation. Followed by one to two hours for pyrosequencing can all be accomplished in a single day.
4.6 Pyrosequencing Results

Notes:

Pyrosequencing results are shown in the next slide. During the validation phase of developing this laboratory developed test, your laboratory will have to develop a library of possible sequence results, and you compare each result with results from your library so that you can interpret whether they’re associated with susceptible or resistant to a particular drug.
4.7 Next Generation Sequencing (NGS)

Next Generation Sequencing (NGS)

- Whole genome sequencing (WGS)
- Targeted amplicon-based sequencing

Notes:

Next I want to talk about next generation sequencing, and these can be either whole genome sequencing where you sequence the entire genome, or you can amplify specific targets within the genome and sequence those.
One example of next generation sequencing is Ion Torrent. This is covered more thoroughly in the molecular biology 101 segment of this series. In Ion Torrent, you do the sequencing during nucleotide synthesis. And the instrument detects changes in pH.
## 4.9 NGS Using Illumina

### NGS Using Illumina

- DNA is fragmented for WGS
- Adapters are added (‘barcodes’)
- 1 molecule is attached to multiple locations on a glass slide
- Molecules are amplified to a localized cluster on the glass slide by ‘bridge amplification’
- Sequencing reaction detects incorporation of sequentially added, fluorescently labeled nucleotides

**Notes:**

Another next generations sequencing method is Illumina. And with the Illumina method, again this is covered more thoroughly in the molecular biology 101, the sequencing is done during synthesis of a nucleotide chain. And the instrument looks for fluorescents as each base is added.
4.10 Considerations for NGS

So let’s look at some considerations about next generations sequencing. It is a rapid assay. Now notice that one of the disadvantages is that it generates massive amounts of data to analyze. This is also an advantage. Massive amounts of data you can look for sequencing of large number of genes and gene sites. It’s possible to have high throughput and it may be cost effective depending on the batch size. However if you delay testing of samples until you accumulate a large batch, this may impact the turnaround time. we don’t know yet whether next generation sequencing will be suitable for testing specimens directly, however it is quite suitable for doing DNA sequencing from TB isolates.
5. Probe-based Molecular Methods

Notes:

In contrast to the sequencing methods we have some commercially available probe-based molecular methods for detecting drug resistance.
5.2 GeneXpert MTB/RIF Assay

Notes:

These include the GeneXpert, MTB/RIF assay, from Cepheid, and this uses five probes, which are molecular beacons, to look for mutations in the *rpoB* rifampin resistance determining region. The molecular beacons are directed against the wild type sequence. So you can look for the presence or absence of the wild type sequence. If the wild type sequence is absent, you can presume that there’s mutation present.
Notes:

Let's take a look at the output from the MTB/RIF, Cepheid GeneXpert instrument. In this slide we notice that at the lower right there's a graph that shows the fluorescents associated with each of the five probes. You'll notice that one of the probes, the green one which is probe B, did not light up. This suggests that there's a mutation in probe B of the \textit{rpoB} gene. So the report here is MTB is detected and rifampin resistance is detected. I think that I should mention at this point sometimes mutations in the probe B region are actual silent mutations, but we're going to talk more about interpretations of Cepheid GeneXpert results in just a minute.
5.4 Considerations for MTB/RIF Assay

Let's talk about some considerations about the MTB/RIF assay from Cepheid, one of the advantages is that it takes place in a self-contained single use disposable cartridge. So that the escape of amplicons from the cartridges is unlikely. And cross contamination among samples is reduced. There are limited biosafety concerns because the sputum sample is mixed with a reagent, which will kill the TB bacteria as well as liquefying the sample before it's added to the test cartridge. It's certainly an advantage that results are available within two hours of a specimen collection. So within two hours within the laboratory, you can get a Cepheid GeneXpert MTB/RIF result.

There are some disadvantages the specific mutations are not identified. So silent mutations could lead to false resistance. And there is the possibility of low positive predicted value in low prevalence populations. So we think that in the United States, where the present of rifampin resistance is low, the confirmatory testing should be done. Also heteroresistance may be missed. Heteroresistance, you do recall, is a mixture of susceptible and resistant bacilli. The molecular beacon probes for the
GeneXpert aren't looking for the wild type sequences. If they're present the GeneXpert will report that they're present and suggest that the TB are susceptible to rifampin. On the other hand if there are a few resistant bacilli as well with mutated sequences, those will not be detected. You notice here that this is suitable for testing specimens or isolates.
5.5 Line Probe Assays

**Line Probe Assays**

- **GenoType MTBDRplus (Hain Lifescience)**
  - Detects presence of MTBC and mutations associated with RIF-R and INH-R

- **GenoType MTBDRsl (Hain Lifescience)**
  - Detects mutations associated with resistance to FQs, second-line injectables, and EMB

- **INNO-LiPA Rif TB (Innogenetics)**
  - Detects presence of MTBC and mutations associated with RIF-R (91% of RIF-R isolates are also INH-resistant)

**Notes:**

Let's talk next about line probe assays. The first of these that's listed is the Genotype MTBDRplus from Hain Lifescience. This is endorsed by the World Health Organization for use in developing countries. It's not yet approved by the US Food and Drug Administration. It detects the presence of TB complex and mutations associated with both rifampin and INH. so this is an advantage compared to the GeneXpert, it can also detect INH resistance.

Then there is the Genotype MTBDRsl, also from Hain. And this can detect mutations associated with resistance to floroquilones, the injectable second line drugs, and ethambutol.

Finally there is the INNO-LiA Rif TB from Innogenetics. And this detects mutations in *rpoB*, so it detects rifampin resistance only and not INH. But 91% of rifampin resistance isolates are also INH resistant from various studies that have been done.
5.6 GenoType MTBDRplus

Notes:

So taking a look at the probes for GenoType MTBDRplus, you'll notice that there are eight probes for the rpoB rifampin resistance determining region, and these are detected by hybridization to probes that are in a line probe.
5.7 MTBDRplus Results

Notes:

Here's an example of some results with a MTBDRplus line probe assay from Hain. You'll notice that on the left it shows that there are wild type probes, for the wild type sequence for the \textit{rpoB} gene for rifampin. The \textit{katG} gene for isoniazid, and the \textit{inhA} gene also for isoniazid. There are also probes for the most commonly occurring mutant sequences for all three of these genes. For line probe number one, you'll notice that all of the wild type sequences show hybridization and this is expected to be a fully susceptible organism. Susceptible to rifampin and isoniazid.

With line probe number two, we see the absence of hybridization to \textit{rpoB} wild type probe seven. And hybridization to \textit{rpoB} mutation probe 2A. This suggests that there's a mutated sequence present which we specifically identified, the sequences, the mutation probe 2A, commonly occurred mutation for \textit{rpoB}. And also with \textit{katG} gene, we see absence of the wild type hybridization probe. Now we see hybridization to \textit{katG} mutation probe one. So this suggests resistance to isoniazid as well.
With probe number three, line probe number three. We see wild type sequences hybridization, for rpoB suggesting susceptibility to rifampin. And we see absence of hybridization to the inhA wild type probe number one. And hybridization to the mutation probe number one, suggesting that there's mutation here in the inhA gene. So we expect it to be resistant to INH.

Now let's skip over to line probe number five. Because this shows a point that I want to make. Here we notice that wild type number seven is not hybridized. So there appears that there is a mutation in this region, the probe seven region. But we don't see hybridization to any of the most commonly occurring mutant probes. So we don't know exactly what the mutation is. There is a possibility that this is a silent or synonymous mutation, or it could be a mutation which is unknown, or one that is not associated with rifampin resistance. So this is interpreted as resistant to rifampin but should be followed up by cultural based, drug susceptibility testing.
5.8 MTBDRsl Results

Notes:

The next slide show results with MTBDRsl, line probe. Again it's from Hain. And this has genes, \textit{gyrA}, \textit{rrs}, and \textit{embB}. And similar to the previous slide, the first culture that's shown for hybridization, the DNA sample hybridized with the wild type probe for \textit{gyrA}, \textit{rrs}, and \textit{embB}, suggesting susceptibility to these three drugs, fluoroquinolones, injectable drugs, and EMB respectively. Looking at line probe number two, we notice an absence of hybridization to \textit{gyrA} wild type probe two.

And the presence of hybridization to \textit{gyrA} mutation probe number one. This suggests fluoroquinolone resistance. Similarly, we have an absence of hybridization to \textit{rrs} wild type probe one, and hybridization with \textit{RRS} mutation probe one suggesting resistance to injectable drugs. And then for \textit{embB}, we see absence of hybridization to the wild type probe, hybridization to the mutation probe 1A, and this suggests resistance to ethambutol.

Now let's move again to the right. And we're going to notice again a problem similar to what we found with the MTBDRplus slide. Sometimes you have absence of
hybridization to the wild type probe for, in this case, gyrA wild type probe one is not hybridizing, but none of the mutation probes are hybridizing either. So there appears to be a mutation in the gyrA wild type probe one region. But we don't know what it is. It's not one of the most commonly occurring mutations, and there is the possibility that it could be a silent or synonymous mutation, an unknown mutation, or one that is not associated with resistance. The MTBDRsl line probe assay has not yet been approved by the World Health Organization.
5.9 Considerations for Line Probe Assays

Notes:

Let's talk about some considerations about line probe assays. They have the advantage that they can work directly on processed specimens, as well as isolates. They're rapid, and they enable analysis and documentation of results.

Among the disadvantages, line probe assays are an open system. It's possible that DNA amplicons can escape then contaminate the laboratory and lead to some false results. Another potential disadvantage for line probe assays, is that silent mutations may result in a false prediction of resistance.
6. Understanding molecular detection of drug resistance

Notes:
Okay let's talk about understanding molecular detection of drug resistance.
6.2 Comparing Molecular-based Assays

Notes:

Let's talk about comparing the molecular based assays. The two on the left, the GeneXpert MTB/RIF, and the Hain GenoType MTBDRplus are commercial systems, of these only the Cepheid GeneXpert has FDA market authorization. The Hain GenoType MTBDRplus can detect INH, as well as rifampin resistance. And they both can be accomplished within one to two working days. On the right the DNA sequencing methods are laboratory developed tests, which require very thorough validation by the laboratory, which is preforming them. This can be quite time consuming, but they have the capability of detecting resistance to first- and second-line drugs by DNA sequencing. And they also can be accomplished within one to two working days.
6.3 Comparing Molecular-based Assays

Notes:

So in summary, comparing molecular based assays, the DNA probe method, like molecular beacons like GeneXpert and line probe assays indicate the presence or absence of a mutation. Now the line probe assays can also identify specific commonly occurring mutations associated with drug resistance, but sequencing provides more detailed information; the detection of specific mutations, and as time goes by we're going to be able to correlate specific mutations and phenotypic drug susceptibility testing, and even quantitative drug susceptibility testing or MICs.
6.4 CDC Guidelines for Use of Xpert MTB/RIF

Next we have a table with CDC guidelines, for the use of Xpert MTB/RIF. And this is one of the more important points that I want to make. I want you to notice that with the GeneXpert it might generate a result that says MTB detected, rifampin resistance detected. What this actually means is that TB is detected in the sample and that there's a mutation in \( rpoB \).

Centers for Disease Control is recommending a different reporting language from what printed out from the GeneXpert instrument. And this is the reporting language, which I would strongly recommend that you use. And it says, “MTB complex is detected. A mutation in \( rpoB \) has been detected indicating possible rifampin resistance. Confirmatory testing should follow.” And I think you know the reason for this, there’s a possibility that the mutation could be a silent or synonymous mutation. Rather than one that confers resistance. The other interpretations are more straightforward, “MTB detected, rifampin resistance not detected,” when there’s no mutation detected in \( rpoB \). You can also get a result from the instrument that says, “MTB detected, and Rif resistance is indeterminate,” and what this means is that two or more of the beacons did not light up, and so it’s unable to determine if there’s an \( rpoB \) mutation. And lastly, MTB not detected and the reporting language that recommend by CDC is the same, “MTBC not detected.”
6.5 Confirmation of Resistance by Xpert MTB/RIF Assay

**Notes:**

So let's talk a minute about confirmation of resistance that's detected by the Xpert MTB/RIF assay. Confirmatory testing using DNA sequencing such be done, including genetic loci associated with MDR TB (\textit{rpoB}, \textit{inhA}, and \textit{katG}) and prompt phenotypic DST.

- Silent mutations in \textit{rpoB} may be detected by MTB/RIF assay, producing a false resistant result.
- Also, mutations that are associated with low-level but clinically relevant RIF-R may be detected; these mutations have been associated with false susceptible results by phenotypic DST.
6.6 Case # 1—Is it RIF-R?

**Notes:**

We've got a couple of cases here, and case number one, the question is, is it rifampin resistant? So in this case the patient has acid fast smear positive pulmonary TB, and the hospital laboratory got some results. The GeneXpert twice reported rifampin resistance detected. And drug susceptibility testing, and the mycobacterial growth indicator tube indicated INH resistant and rifampin susceptible. So here there's a discrepancy, the Xpert is saying resistance detected, and the cultural based testing says rifampin susceptible. The agar proportion drug susceptibility testing is pending at the public health lab. And so it's referred to a laboratory that does sequencing of the *rpoB* gene, and they find a Phe 514 Phe silent mutation. So is this rifampin resistant? Or not?
Case # 1—Is it RIF-R? (2)

• Silent mutations in RDDR of \( rpoB \) generally not associated with RIF-R
• Xpert detected the silent mutation and the isolate is not RIF-R
• Sequencing was required to determine the cause of the discordance

Notes:

So taking a look at the next slide. We're reminded that the silent mutations in the rifampin resistance determining region of \( rpoB \) are generally not associated with rifampin resistance. Xpert detected the silent mutation but the organism is not really rifampin resistant. So sequencing was required to determine the cause of the discordance and clarify the situation. This is one of the reasons why we recommend DNA sequencing.
So let's take a look at case number two. Here the public health laboratory has some drug susceptibility testing results. In MGIT the isolate is resistant to INH and susceptible to rifampin at the usual test concentration of one microgram per milliliter, but with agar proportion testing, we have resistant to INH and resistant 80 percent resistant to rifampin at the same test concentration. So this was referred for pyrosequencing. What pyrosequencing found was that there was a mutation in the \textit{rpoB} that caught on 516, which is associated with rifampin resistance. And there was a mutation in \textit{inhA} gene causing INH resistance as expected. Agar proportion testing at a reference laboratory found rifampin resistance with 40 percent resistance by the agar proportion. So there was an observed discordance between broth and agar phenotypic drug susceptibility testing. So what do we think about this one?
6.9 Case # 2—Is it RIF-R? (2)

Notes:

Okay for case number two, the \( rpoB \) mutation detected is associated with highly discordant drug susceptibility testing results. Also called low-level or borderline resistance. Resistance of this kind is probably clinically relevant. And this kind of resistance is often missed by standard growth-based systems like the mycobacterial growth indicator tube.

Possibly the critical concentration in the mycobacterial growth indicator may be too high to enable detection of all clinically relevant resistance. So the method may need some modification in the future. We don't know what kind of modification could correct this problem. Perhaps longer incubation or larger inoculum size to detect resistance. The prevalence with these strains with low level or borderline resistance mutations is unknown. And some examples are the mutations that are listed there in codons 516, 511, 533, and 526. As well as 572 of the \( rpoB \) gene.
Next let's talk about communicating molecular results.
7.2 Reporting Molecular Results

Notes:

When we're reporting molecular results they need to be provided in a format that is clear and understandable to the healthcare provider. Keep in mind that because these are new technologies, education and training of healthcare providers is needed. So that they can understand the test results and limitations. We need to read the package inserts, so that we understand the reporting language and its interpretations. And to be included in the report, we should consider what test was performed, the method that was used, and a comprehensive result. For example, the mutation including the nucleotide sequence and amino acid substitution, when that's applicable, not just, “mutation detected or not detected,” if the actual sequence is available. However if you're using Cefeid GeneXpert you can just report, “mutation detected or not detected.” And it's helpful if the report provides interruptive comments.
Help is available for education and training of healthcare providers, from regional training and medical consultation centers. You can see from this map that there are five of these. And there's a web link listed at the bottom. If you go to this web link, you can find out the specific contact information for each of the regional training and medical consultation centers. These are assigned to cover all 50 states and territories. And they provide medical consultation to TB programs and medical providers.
7.4 Need for a Drug Resistance Database

Notes:

We need to have a drug resistance database. It's going to take a long time to develop this. We need continued progress and tools for rapid detection of drug resistant tuberculosis. And we need to improve the understanding of the molecular basis of drug resistance and the clinical relevance of each type of mutation. This effort likely will require a comprehensive and curated data platform. Curated so that it contains only accurate information. We'll give the correlation between sequencing data and phenotypic drug susceptibility, as well as clinical significance. A database of this kind could inform diagnostics development and clinical decision making using molecular results.
7.5 Resources

Notes:

The next slide contains references and resources associated with this module, and it can be found on the APHL website.
7.6 Journal Articles of Interest


Notes:

The next slide contains references and resources associated with this module, and it can be found on the APHL website.
7.7 Journal Articles of Interest-2


- Lin, S.Y.G., and Desmond, E.P. (2014). “Molecular Diagnosis of Tuberculosis and Drug Resistance” Microbial Diseases Laboratory, California Department of Public Health


Notes:

The next slide contains references and resources associated with this module, and it can be found on the APHL website.
7.8 Journal Articles of Interest-3

Journal Articles of Interest-3


Press next for more Resources

Notes:

The next slide contains references and resources associated with this module, and it can be found on the APHL website.
7.9 Journal Articles of Interest-4


Notes:

The next slide contains references and resources associated with this module, and it can be found on the APHL website.
7.10 Thank You for Participating!

This concludes the Molecular Detection of Drug Resistance presentation, which is part of the series, Essentials for the Mycobacteriology Laboratory, Promoting Quality Practices. Please see the CDC and APHL websites for more information on these topics.