Drug Susceptibility Testing for *M. tuberculosis* Complex
Presentation Overview

• Background on drug resistance
• Conventional drug susceptibility testing (DST) methods
• DST turnaround times and reporting
• Discordance in DST results
• Ensuring quality
• Brief description of molecular methods for resistance detection
Drug Susceptibility Testing for *M. tuberculosis* complex

BACKGROUND ON RESISTANCE
Drug Resistant TB

• Multidrug resistant tuberculosis (MDR TB)—resistant to at least rifampin (RMP) and isoniazid (INH)

• Extensively drug resistant TB (XDR TB)—MDR TB plus resistance to at least 1 fluoroquinolone (FQ) and 1 second-line injectable drug (SI)
TB Treatment Regimens

• Pulmonary, drug susceptible TB, 6-month standard regimen
  – Initial phase: 2 months INH, RMP, ethambutol (EMB), and pyrazinamide (PZA)
  – Continuation phase: 4 months of INH and RMP

• MDR TB
  – Usually at least 3 previously unused drugs to which isolate is susceptible and then individualize

• XDR TB
  – Individualized therapy
MDR TB Treatment

• Approximately 2 years of therapy with a combination of first and second-line drugs
• Second-line drugs include FQ (e.g., ofloxacin, ciprofloxacin, moxifloxacin, levofloxacin), SI (i.e., capreomycin, amikacin, and kanamycin), PAS, ethionamide, cycloserine, and bedaquiline
• Regimen can become very complex depending on the extent of additional resistance beyond RMP and INH
• Second-line drugs often cause severe adverse effects and may be difficult for patients to tolerate
First-line Drugs and Mechanisms of Action

Mycobacterium tuberculosis

- **Isoniazid (1952)**
  - Inhibits cell wall synthesis

- **Ethambutol (1961)**
  - Inhibits cell wall synthesis

- **Pyrazinamide (1952)**
  - Exact Target Unclear
  - Disrupts Plasma Membrane
  - Disrupts Energy Metabolism

- **Rifampin (1966)**
  - Inhibits RNA synthesis

**Cell Wall Synthesis**
- Acyl Lipids
- Mycolic Acid
- Arabinogalactan
- Peptidoglycan
- Plasma Membrane

**DNA Coiling, Transcription, and Translation**
- RNA Polymerase
- DNA Gyrase
- mRNA
- Ribosome
- Protein

**ATP Synthesis**
- ATP

National Institute of Allergy and Infectious Diseases (NIAID)
Intrinsic Drug Resistance

• Hydrophobic cell envelope (permeability barrier)

• Drug efflux systems and drug modifying enzymes
  – Efflux systems pump toxic substances out of cell and enzymes to change the drug configuration

• Intrinsic PZA resistance seen with some members of the *M. tuberculosis* complex (MTBC) due to lack of pyrazinamidase activity
  – *M. canetti*, *M. bovis* and *M. bovis* BCG (other members of MTBC are usually susceptible to PZA)
Selection of Drug Resistant Mutants in TB

• Spontaneous mutations occur in the DNA of all cells
  – Mutations can change the structure of a protein that is a drug target
  – Protein still functions, but is no longer inactivated by the drug
  – Thus, TB can grow in the presence of the drug

• Resistance is linked to large bacterial populations
  – Mutants resistant to any drug naturally occur on average once in every 100 million \((10^6–10^8)\) cells
  – Pulmonary TB—cavities often contain \(10^7–10^9\) organisms
  – By using two antibiotics, 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
Spontaneous mutations develop as bacilli proliferate to $>10^8$

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mutation Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP</td>
<td>$10^{-8}$</td>
</tr>
<tr>
<td>INH</td>
<td>$10^{-6}$</td>
</tr>
<tr>
<td>PZA</td>
<td>$10^{-6}$</td>
</tr>
</tbody>
</table>
Drug-resistant mutants in large bacterial population

Multidrug therapy: No bacteria resistant to all 3 drugs

Monotherapy: INH-resistant bacteria proliferate
Spontaneous mutations develop as bacilli proliferate to $>10^8$

INH resistant bacteria multiply to large numbers

INH mono-resistant mutants killed, RMP-resistant mutants proliferate $\rightarrow$ MDR TB
Other Factors Influencing Development of Drug Resistance

- Treatment with inappropriate drugs, combinations or dosages
- Interrupted or irregular treatments
- Incomplete treatments
  - Duration—patient stops early because feels better
  - Required number of doses not taken (patient non-compliant)
- Metabolism of bacilli shifted to dormancy
  - Impaired/ decreased drug uptake by *M. tuberculosis* cell
- Penetration of drugs to various body sites
  - Some bacilli are within macrophages or other cells
  - Suboptimal drug concentration at some body sites
- Impaired drug absorption due to underlying host conditions such as HIV/AIDS, diabetes
Primary vs. Acquired Drug Resistance

- **Primary**—Strain is drug resistant at start of treatment, patient never treated in past
  - Implies transmission of drug-resistant bacilli

- **Acquired**—Strain is drug susceptible at start of treatment, becomes drug resistant during treatment
DST of MTBC is Essential

- Guides choice of chemotherapy—provides the best chance of cure
- Detects drug resistance or confirms the emergence of drug resistance when a patient fails to show a satisfactory bacteriologic response to treatment; guides the choice of treatment with different drugs
- Offers insight into appropriate treatment for contacts of patients with active TB
- Used to estimate the prevalence of primary and acquired drug resistance in a community
Drug Susceptibility Testing for *M. tuberculosis* complex

CONVENTIONAL METHODS
Critical Concentration

- DST of MTBC typically involves testing the susceptibility of the organism against the critical concentration of a drug
- Critical concentrations were adopted by international convention
- By definition, the critical concentrations represent the lowest concentrations of drugs that inhibit 95% of wild-type strains that have never been exposed to antituberculous drugs, while at the same time not inhibiting growth of strains that have been isolated from patients who are not responding to therapy and are considered resistant
Critical Concentration

• Ideally the critical concentration is the lowest concentration of a drug that discriminates between susceptible and resistant strains of MTBC
  – Inhibits growth of all susceptible strains
  AND
  – Allows growth of all resistant strains

• It is difficult to find a drug concentration that precisely meets this definition; we settle for the concentration that **BEST DISCRIMINATES** between susceptibility and resistance
Determining Critical Concentrations

RMP

INH

Difficulty of Determining Critical Concentrations

Equivalent Concentrations

- Critical concentrations were originally determined in Lowenstein-Jensen medium
- Equivalent concentrations of drugs later established in Middlebrook 7H10 and 7H11 for agar proportion method and in media used in commercial DST systems
Recommended Equivalent Test Concentrations for First-line Drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>System and Concentration (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7H10 Agar</td>
</tr>
<tr>
<td>INH low</td>
<td>0.2</td>
</tr>
<tr>
<td>INH high</td>
<td>1.0</td>
</tr>
<tr>
<td>RMP</td>
<td>1.0</td>
</tr>
<tr>
<td>EMB</td>
<td>5.0</td>
</tr>
<tr>
<td>PZA</td>
<td>-</td>
</tr>
</tbody>
</table>
# Recommended Concentrations for Second-line Drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>System and Concentration (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7H10 Agar</td>
</tr>
<tr>
<td>Amikacin</td>
<td>4.0</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>5.0</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>10.0</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>2.0</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>1.0</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.5</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>5.0</td>
</tr>
<tr>
<td>PAS</td>
<td>2.0</td>
</tr>
<tr>
<td>Rifabutin</td>
<td>0.5</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>2.0 and 10.0</td>
</tr>
</tbody>
</table>

*Most concentrations listed are based on multicenter studies. Systems are not cleared by the FDA for testing second-line drugs (except Streptomycin)*
Under some circumstances, laboratories may perform tests to establish a minimum inhibitory concentration (MIC) for each antituberculous drug. The MIC is determined as the lowest concentration of a series of drug dilutions that prevents visible growth of MTBC in a broth dilution DST. This differs from testing using critical concentrations which uses single drug concentrations and provides a categorical result of resistant or susceptible.
Recommended Panel for DST

- Initial MTBC isolates from ALL patients should be tested for susceptibility against four first-line drugs
  - INH, RMP, EMB, and PZA

- Isolates resistant to RMP or any two first-line drugs, should be tested against second-line drugs
  - Minimally, second-line panel should include at least one FQ, amikacin, kanamycin, and capreomycin

- DST should be repeated after 3 months if patient remains culture positive
DST Should Always be Performed on a Pure Culture

- Indirect DST is performed after growth is identified as MTBC.
- It is essential to ensure that MTBC cultures are pure; contaminating bacteria are known to harbor intrinsic resistance and potentially cause false-resistant MTBC DST results.
- It is recommended that broths also be sub-cultured to 7H10/7H11 and blood agar to assess purity and colony morphology.
- If a culture is mixed with NTM or other bacteria, laboratories can attempt to re-isolate the MTBC.
## Culture-based Methods for DST

<table>
<thead>
<tr>
<th></th>
<th>MGIT 320 or 960</th>
<th>VersaTREK</th>
<th>Indirect Agar Proportion</th>
<th>Sensititre</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Company</strong></td>
<td>Becton Dickinson</td>
<td>Thermoscientific</td>
<td>N/A</td>
<td>Thermoscientific</td>
</tr>
<tr>
<td><strong>Media</strong></td>
<td>Liquid broth</td>
<td>Liquid broth</td>
<td>Solid</td>
<td>Liquid broth</td>
</tr>
<tr>
<td><strong>Format</strong></td>
<td>Tube</td>
<td>Tube</td>
<td>Petri plate</td>
<td>96-well microtitre plate</td>
</tr>
<tr>
<td><strong>FDA approved</strong></td>
<td>Yes (cleared)</td>
<td>Yes (cleared)</td>
<td>No (laboratory developed test)</td>
<td>No (research use only)</td>
</tr>
</tbody>
</table>
Procedure for Agar Proportion DST

• Agar proportion (AP) is performed when a bacterial suspension of known concentration (density equivalent to 0.5 to 1 McFarland turbidity standard) is spread onto solid media containing
  – No drugs (growth control)
  – Critical concentrations of first-line and second-line antituberculous drugs
  – PZA cannot be tested using agar proportion method
    • 7H10/7H11 do not support the low pH conditions required for PZA testing

• Incubate inoculated plates for 3 weeks after sealing

• Observe and enumerate the number of colonies each week

Isolate is resistant if the number of colonies on drug-containing media is \( \geq 1\% \) of the colonies on drug-free media
Analysis of Results from Agar Proportion Method

**Growth control (no drug) quadrant**
90 colonies

**INH quadrant**
30 colonies
INH $30 / 90 = 33\%$ resistant

**RMP (R) quadrant**
23 colonies
RMP $23 / 90 = 25\%$ resistant

**Streptomycin (S) quadrant**
No colonies = susceptible
Commercial Broth Systems

• Selection of critical concentrations based on comparison of results with agar proportion to determine equivalent critical concentrations

• More rapid turnaround time results (4-13 days) than agar proportion (21-28 days)

• Continuous, automated signal detection (e.g., fluorescence for MGIT, pressure changes for VersaTREK) by instrument reducing hands-on time

• Published evaluations of second-line drugs (no FDA-cleared methods)
# Commercial Broth Systems

<table>
<thead>
<tr>
<th></th>
<th>MGIT 320 or 960</th>
<th>VersaTREK</th>
<th>Sensititre</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Signal detected</strong></td>
<td>Fluorescence released and detected due to oxygen consumption by growing microorganisms</td>
<td>Pressure changes in headspace of the bottle from oxygen consumption by growing microorganisms</td>
<td>Plates are examined for growth either manually using view box or using the Vizion system</td>
</tr>
<tr>
<td><strong>Turnaround times</strong></td>
<td>Can be inoculated from either liquid or solid media and results available 4-13 days later</td>
<td>Can be inoculated from either liquid or solid media and results available 4-13 days later</td>
<td>Plates are inoculated from growth on solid media and can be read 10-14 days later</td>
</tr>
<tr>
<td><strong>Results:</strong> Susceptible, Resistant or MIC</td>
<td>Usually Susceptible/Resistant-</td>
<td>Susceptible/ Resistant</td>
<td>MIC- laboratory might provide interpretive criteria of Susceptible/Resistant</td>
</tr>
<tr>
<td><strong>Drugs</strong></td>
<td>INH, RMP, PZA, EMB, and sometimes Streptomycin (other drugs available as laboratory developed tests)</td>
<td>INH, RMP, PZA, EMB</td>
<td>INH, RMP, EMB, streptomycin, Rifabutin, Ethionamide, Amikacin, Kanamycin, Ofloxacin, Moxifloxacin, Cycloserine, PAS</td>
</tr>
</tbody>
</table>
Broth-based DST
MGIT 320/960

0.5 mL of organism suspension diluted 1:100

Growth Control

INH low
INH high
RMP
EMB

0.5 mL of organism suspension undiluted
Alternative Inoculum Preparation for MGIT

• Becton Dickinson package insert for preparing inoculum for DST in MGIT includes a protocol for preparing inoculum from solid medium
  – Suspensions are made, allowed to settle, and supernatants diluted to turbidity equivalent to 0.5 McFarland
  – Further 1:5 dilution is made for inoculating drug-containing tubes; growth control is 1:100 dilution of the 0.5 McFarland
  – Lin, et al. (2009) described a similar method for preparing inoculum from MGIT broth, based on turbidity
  – Improved reproducibility was reported (JCM 47:3630)
VersaTREK

- PZA tested at 300 mcg/ml
- Bottles can be inoculated by pipette or needle
- Same system used for mycobacteria growth and detection from sputum, sterile body fluids, and blood can be used for DST
- Sophisticated LIMS interface
- Instrument configurations holding 240 or 528 bottles available
Confirming Resistance from Broth Systems

- Examine growth from purity plate to check for contaminating organisms
- Examine growth from the drug-containing vial to determine consistency
  - Clumps vs. perfuse turbidity?
- Prepare a smear from the vial/tube
  - Examine for cording, dispersed distribution, random clumps
- Subculture vial/tube to 7H10/7H11 solid media to examine colony morphology
- Repeat test from pure culture or different isolate from the same patient if smear of growth indicates a mixed or contaminated culture
Sensititre MYCOTB MIC Plate

- 96-well microtiter plate containing a panel of 12 first and second-line antituberculous drugs
- Plate contains a minimum of 7 dilutions per drug
- A bacterial suspension of known concentration (1X10^5 cfu/ml) is prepared and inoculated into wells of the plate
- Growth can be examined manually using a view box or the Sensititre Vizion® System
- Resistant results can be detected in as little as 7–10 days
Growth in MYCOTB MIC Plate

OFL  MXF  RMP  AMK  STR  RFB  PAS  ETH  CYC  INH  KAN  EMB
Limitations of MIC Testing for MTBC

- Procedures are not standardized
- Assays not FDA-cleared
- No universally established breakpoints or interpretive criteria
- Few studies on how MIC correlates with clinical presentation or patient outcome
- MIC results may not correlate with results obtained by critical concentration methods
- Additional research is needed to understand how DST results using different methods correlate with treatment efficacy
Drug Susceptibility Testing for *M. tuberculosis* complex

TURNAROUND TIME AND REPORTING
Although indirect agar proportion is considered the standard method for DST, it is not a rapid method.

Initial isolates of MTBC should be tested against a panel of first-line drugs using a rapid commercial broth system.

CDC recommends that DST results should ideally be available to the submitter within 28 days of specimen receipt.

- Meeting the recommended turnaround time necessitates the use of a rapid commercial broth system.
Concerns with Current DST Practices

• Most laboratories refer for DST with multiple referrals needed for a full panel of first and second-line antituberculous drugs
• Laboratories may lack confidence in or be reluctant to report resistance prior to confirmation
• Discordant results can occur within or among laboratories and methods
• Manpower and training issues as expertise is required
Considerations for Reporting DST Results

• Any resistance should be considered a critical value and the submitter and public health authorities should be notified immediately

• Issue preliminary reports as results become available
  – Not necessary to wait for all first-line DST results before issuing a preliminary report
    • For example, RMP, INH, and EMB DST may be complete even though PZA results may be pending
Considerations for Reporting DST Results (2)

• If resistance detected, issue preliminary report describing results while concurrently confirming resistance and requesting DST with second-line drugs
  – Also indicate the test is being repeated
  – If resistance is confirmed, issue final report

• For patients with resistant isolates, consultation with reference laboratory and specialists in the management of drug resistant TB should be considered

• If the resistance does not confirm
  – Call provider to inform of discordant results
  – Issue a second preliminary report
  – Submit to CDC or other reference laboratory for testing by the same or different method (e.g., agar proportion)
Conventional DST Report

• Reports should, at minimum, include the name of the drug tested and a clinically relevant interpretation such as susceptible or resistant.

• If reports indicate the concentrations tested for each drug, it should also include the testing medium and/or method used.

• If agar proportion is used, the percent resistance may also be reported.

• If more than one concentration is tested for a drug, interpretive comments may be included on reports (e.g., high and low concentrations of INH).
Considerations for DST Referral Process

• Submitting and referral laboratories should be familiar with shipping guidelines for infectious substances

• If possible, laboratories should refer liquid cultures for DST rather than waiting for growth on solid media

• Submitting laboratories should routinely monitor turnaround time of the referral laboratory
Drug Susceptibility Testing for *M. tuberculosis*

**DISCORDANT RESULTS**
Discordant DST Results

• Can occur among different laboratories, different DST methods and within the same method
• What do discordant results indicate?
• Which result is correct?
  – All
  – Only one
  – None
Reasons for Discordant DST Results

• Differences in bacterial population (original isolate versus subculture)
  – Different inoculum (e.g., isolates from different specimens; sampling from same specimen)
• Differential growth kinetics
• Varying inoculation methods
• Another method
• Different media components (e.g., OADC)
Reasons for Discordant DST Results (2)

• Operator or laboratory error
  – Deviation from standard protocol
  – Transcription, labeling errors

• Cross-contamination

• Variability of isolate in that the MIC is close to the critical concentration tested

• Difficult drugs and lack of standardized methods for second-line DST
Standardization and Reliability Issues

• Some drugs can be difficult to test
  – EMB
    • Can result in microcolonies on agar proportion due to the bacteriostatic nature of the drug
    • Data suggest false susceptibility may be of concern in commercial broth systems
  – PZA
    • Requires testing in acidified liquid media that can impact growth
    • Sensitive to over inoculation resulting in false resistance
    • Data suggest false resistance may be of concern in commercial broth systems
Standardization and Reliability Issues (2)

- Some drugs can be difficult to test
  - Cycloserine
    - Important second-line therapeutic option
    - Cannot be reliably tested by methods available in United States

- Data regarding optimal testing of second-line drugs are limited and standardized protocols require additional evaluation
## Variability in DST Results for EMB

**EMB results reported by MPEP participants for six isolates of EMB-resistant MTBC, 2009–2010**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>embB mutation</th>
<th>CDC AP (％ R)</th>
<th>MPEP Participant Results (No. reported Resistant) / (No. reported results) [% reported Resistant]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AP</td>
<td>MGIT</td>
</tr>
<tr>
<td>2009P</td>
<td>Glu378Ala</td>
<td>100%</td>
<td>21/21 [100]</td>
</tr>
<tr>
<td>2010A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Gly406Asp</td>
<td>40%</td>
<td>9/22 [41]</td>
</tr>
<tr>
<td>2010E&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Gly406Asp</td>
<td>40%</td>
<td>9/22 [41]</td>
</tr>
<tr>
<td>2010C&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Met306Val</td>
<td>100%</td>
<td>23/26 [88]</td>
</tr>
<tr>
<td>2010D&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Met306Val</td>
<td>100%</td>
<td>24/26 [92]</td>
</tr>
</tbody>
</table>

<sup>a</sup> same strain; <sup>b</sup> same strain
## Variability in DST Results for PZA

<table>
<thead>
<tr>
<th>Isolate*</th>
<th>Bactec 460</th>
<th>MGIT</th>
<th>VersaTREK</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0/17 (0)</td>
<td>1/64 (2)</td>
<td>0/5 (0)</td>
</tr>
<tr>
<td>B</td>
<td>0/17 (0)</td>
<td>7/62 (11)</td>
<td>0/3 (0)</td>
</tr>
<tr>
<td>C</td>
<td>0/17 (0)</td>
<td>20/62 (32)</td>
<td>3/3 (100)</td>
</tr>
<tr>
<td>D</td>
<td>0/17 (0)</td>
<td>21/63(33)</td>
<td>3/3 (100)</td>
</tr>
<tr>
<td>E</td>
<td>0/17 (0)</td>
<td>0/64 (0)</td>
<td>(0)</td>
</tr>
</tbody>
</table>

*A and E are same strain; C and D are same strain.
Bactec 460 system no longer commercially available.

- Data indicate potential false PZA resistance in some automated liquid systems.

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Data from 2010 CDC Model Performance and Evaluation Program for MTBC DST
When PZA Testing is Likely to be Repeated

- MGIT readouts
  - Error (X) (e.g., failed to grow or grew too fast)
  - “Low Resistant” result (e.g., 400/150)
  - “High Susceptible” result (e.g., 400/84)
- Discordance with referring laboratory’s result
- PZA monoresistance (not known to be *M. bovis*)
- Susceptible in MGIT, but mutation detected in *pncA* gene by molecular testing
- Resistant in MGIT, but no mutation detected in *pncA* gene by molecular testing
Considerations for Detecting RMP Resistance

• Level of resistance to RMP can vary depending on specific mutations present within \textit{rpoB}

• Reports of low-level but presumably clinically significant RMP resistance being missed by commercial broth systems
  – Can result in discordance between conventional and molecular test methods

• Concern that missing RMP resistance through conventional methods could impact clinical outcomes for patients
### Variability in DST Results for RMP

<table>
<thead>
<tr>
<th>Method</th>
<th>Strain H (6/2008)*</th>
<th></th>
<th>Strain T (5/2010)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. RMP-Resistant/No. results</td>
<td>%</td>
<td>No. RMP-Resistant/No. results</td>
</tr>
<tr>
<td>Agar Proportion</td>
<td>19/27</td>
<td>70</td>
<td>15/23</td>
</tr>
<tr>
<td>BACTEC 460</td>
<td>15/36</td>
<td>41</td>
<td>7/19</td>
</tr>
<tr>
<td>MGIT</td>
<td>13/69</td>
<td>18</td>
<td>9/61</td>
</tr>
<tr>
<td>VersaTREK</td>
<td>0/3</td>
<td>0</td>
<td>0/5</td>
</tr>
<tr>
<td>Total</td>
<td>54/139</td>
<td>38</td>
<td>31/108</td>
</tr>
</tbody>
</table>

*His526Leu mutation in \( rpoB \)

Data from CDC Model Performance Evaluation Program
Drug Susceptibility Testing for *M. tuberculosis* complex

ENSURING QUALITY
Quality Control

• Quality Control (QC) for DST should include an isolate that is susceptible to all drugs being tested (e.g., H37Rv)

• Although not necessary, laboratories may choose to test a resistant strain as part of their QC

• Use of a single strain that is resistant to more than two drugs is not recommended due to safety risk

Reference: CLSI M24A2
Quality Control (2)

- **Drug susceptible QC strain should be run**
  - For each new lot of drug or media component before use on patient isolates
    - Especially for Oleic Albumin Dextrose Catalase (OADC) and 7H10 powder
  - Each time new batch of media is prepared
  - At least once a week or with each run

- **If a resistant strain is used for QC, it may be tested less frequently than drug susceptible strain**
Quality Control (3)

• Failure occurs when drug susceptible QC strain does not grow in the growth control (i.e., no drug) or exhibits growth in drug(s) being tested
• Patient results for the drug or drugs that failed QC should not be reported for that testing period
• Testing for drugs and patient isolates affected by the QC failure should be repeated
• Most common causes of MGIT DST QC failure include contaminated QC cultures, over or under inoculated cultures, no drug added to tubes, and instrument errors
• Growth in drug-containing (resistant) tubes should be checked for purity using Ziehl Neelsen or Kinyoun stain of smear
Drug Resistance Rates as a Performance Indicator

- True rate is dependent on population or geographic region
  - Decrease or increase may be due to shift in patient population
- False increase may be due to
  - Cross contamination during specimen collection or processing
  - Mixed cultures (NTM or contamination)
  - Inappropriate drug concentrations or media preparation
  - Errors in interpretation
- False decrease
  - Inappropriate drug concentrations or media preparation
  - Errors in interpretation
- Do not assume that a drug resistant isolate is due to contamination
Proficiency Testing and Evaluation Programs for DST of MTBC

• Maintaining technical proficiency is critical to ensure rapid and reliable DST results
• Low volume laboratories processing fewer than 50 isolates/year should refer to laboratories with demonstrated technical proficiency for DST
• Formal proficiency testing for first-line DST is available through the College of American Pathologists
• CDC manages the voluntary Model Performance Evaluation Program
  http://www.cdc.gov/tb/topic/laboratory/mpep/
  – Program provides challenges to assess detection of first and second-line resistances
Drug Susceptibility Testing for *M. tuberculosis* complex

MOLECULAR METHODS
Use of Molecular Assays to Detect Resistance

- Conventional DST requires growth of the organism and turnaround time is measured in weeks.
- Molecular assays to detect mutations associated with resistance reduce turnaround time to hours or days.
  - Provide more timely guidance for clinical management, especially where there is risk of drug resistance or resistance is suspected.
- Molecular assays may require an isolate or use a clinical specimen as the sample material.
- Conventional DST still required due to gaps in knowledge and limit of detection issues.
- Under some circumstances, molecular results may be more accurate (e.g., mutations resulting in low level but clinically significant RMP resistance).
## Examples of Tests for Molecular Detection of Mutations Associated with Drug Resistance

<table>
<thead>
<tr>
<th>Company</th>
<th>GeneXpert® MTB/RIF</th>
<th>HAIN Genotype® MTBDRplus</th>
<th>Sanger Sequencing</th>
<th>Pyrosequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company</td>
<td>Cepheid</td>
<td>HAIN Lifescience</td>
<td>Not Applicable (N/A) (laboratory developed test)</td>
<td>N/A (laboratory developed test)</td>
</tr>
<tr>
<td><strong>Genetic loci</strong></td>
<td>rpoB (for RMP)</td>
<td>rpoB (RMP), katG (INH), and inhA (INH)</td>
<td>Varies but can include rpoB, inhA, katG, aphC, embB (EMB), pncA (PZA), gyrA (FQ), and rrs (injectables)</td>
<td>Varies but can include rpoB, inhA, katG, aphC, gyrA, and rrs</td>
</tr>
<tr>
<td><strong>Format</strong></td>
<td>Semi-automated real-time PCR</td>
<td>Line probe assay</td>
<td>DNA sequencing</td>
<td>DNA sequencing</td>
</tr>
<tr>
<td><strong>FDA approved</strong></td>
<td>Market authorization</td>
<td>No</td>
<td>N/A (laboratory developed test)</td>
<td>N/A (laboratory developed test)</td>
</tr>
<tr>
<td><strong>Expected turnaround time from receipt in laboratory</strong></td>
<td>1-2 working days</td>
<td>1-2 working days (depends on how often performed in lab)</td>
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</tbody>
</table>