National TB DST Reference Center Update

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Why do we need a DST ref center?

• Decreasing TB cases
  – More than 20 states have TB case-load below 50.
• Complex test procedures
• Ever-advancing technologies
• When tests are infrequently performed, it is hard to maintain competency and proficiency
• Services provided by the DST Ref Center:
  – Molecular detection of drug resistance
  – Culture-based DST (or “growth”-based DST)
Program Overview

• Funded, structured and monitored by CDC/APHL.
• Eligible to states with annual TB case load <50.
• Enrollment is simple; contact Will Murtaugh at APHL.
  • Phone: 240.485.2764  <william.murtaugh@aphl.org>
• More info at www.APHL.org
• Tests offered at the ref center (MDL)
  – MDST by pyrosequencing (PSQ): performed daily.
  – CDST by MGIT 960: performed 3 times/week.
• Reports are faxed to submitting labs.
• Genotyping: isolates are forwarded to MI.
Current Status

• 22 states are eligible
• 13 states signed up
• 1\textsuperscript{st} specimen rec’d on 3/6/15 from Wyoming.
• So far 54 specimens rec’d from 8 states.
PSQ: rapid detection of DR

• Pre-approval required.
  – Send requests to CDPHTBDST@cdph.ca.gov
  – Acceptance criteria: DR suspected; Pt not responding to treatment; mixed or contaminated cultures, etc.

• Specimens
  – Smear-positive sediments
  – Positive cultures

• Turnaround time
  – 1-3 days (Median: 1 day).

• CDST by MGIT will follow.
  – No mutations, test 1\textsuperscript{st}-line drugs.
  – Mutations detected, test 1\textsuperscript{st}-line & 2\textsuperscript{nd}-line drugs.
PSQ

• **INH:** *katG*, promoters of *inhA & ahpC, and fabG1*
  - Sensitivity: 88%. NPV: >98% (INH-R rate at 10%)
  - Specificity: 100%. PPV: 100%

• **RIF:** *rpoB* (codons 507 to 533, and 176)
  - Sensitivity: 97-98%
  - Mutations are not equivalent; they confer different levels of RIF-resistance; some do not confer phenotypic resistance.
  - MDL has RIF MIC data for 47 mutations detected.
  - Silent mutations—do not confer RIF-R; frequently detected.
    - Of all *rpoB* mutations detected at MDL, 20% are silent mutations.
    - Xpert does not distinguish silent mutations from other mutations.
      - Leads to wrong interpretations. Must be sequenced to obtain mutation ID.
      - Watch out for probe B; 514TTT—most common!
Pyrogram reveals NT sequences

<table>
<thead>
<tr>
<th>Sample ID:</th>
<th>H37RV</th>
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<tbody>
<tr>
<td>Well:</td>
<td>E9</td>
</tr>
<tr>
<td>PSQ run:</td>
<td>05_24_13_ALL_SL</td>
</tr>
<tr>
<td>Entry ID:</td>
<td>r8-S1-507-521-021413</td>
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</tbody>
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Query sequence: GGCACCAGCCAGCTGAGCCATTCATGGACCAAGAACACCCGCTG

Result: RA01, rpoB1 no mutations within 507-521
Quality: Good
Information: Low score discrimination between best and second best hit.

### Hit 1: RA01, rpoB1 no mutations within 507-521
- Score: 100
- Identities: 45/45 (100%)
- Gaps: 0/45 (0%)
- Query: GGCACCAGCCAGCTGAGCCATTCATGGACCAAGAACACCCGCTG 45
- Library: GGCACCAGCCAGCTGAGCCATTCATGGACCAAGAACACCCGCTG 45

### Hit 2: RA01-06, rpoB1 no mutations within 507-521,(4C misread at 520)
- Score: 97.2
- Identities: 45/46 (98%)
- Gaps: 1/46 (2%)
- Query: GGCACCAGCCAGCTGAGCCATTCATGGACCAAGAACACCCGCTG 45
- Library: GGCACCAGCCAGCTGAGCCATTCATGGACCAAGAACACCCGCTG 45

### Hit 3: RA01-02,rpoB1 no mutations within 507-521,(2C misread at 519)
- Score: 97.2
- Identities: 44/45 (98%)
- Gaps: 1/45 (2%)
- Query: GGCACCAGCCAGCTGAGCCATTCATGGACCAAGAACACCCGCTG 45
- Library: GGCACCAGCCAGCTGAGCCATTCATGGACCAAGAACACCCGCTG 45
Wildtype, \textit{rpoB} 514 TTC

A silent mutation, \textit{rpoB} 514 TTT
CDST

• Culture-based DST by MGIT 960
  – 1\textsuperscript{st}-line: RIPE
  – 2\textsuperscript{nd}-line: MACE, will add KAN after validated

• Reflexed 2\textsuperscript{nd}-line DST
  – When R to any 1\textsuperscript{st}-line drugs.
  – When mutations are detected by PSQ

• Reflexed PSQ
  – When cultures are contaminated.
  – When cultures are mixed with NTM.
  – When cultures grow too slowly, or DST fails.
  – Quick confirmation of R by CDST.
    • Rule in R when a mutation detected.
    • Repeat CDST when mutations not detected.
## Contact list

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<thead>
<tr>
<th>Name</th>
<th>Email account</th>
<th>Phone/Fax</th>
<th>Functions</th>
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<tbody>
<tr>
<td>TB Ref lab</td>
<td><a href="mailto:CDPHTBDST@cdph.ca.gov">CDPHTBDST@cdph.ca.gov</a></td>
<td>510-412-3949 (phone)</td>
<td>Request for PSQ approval Notification of DST submission General info</td>
</tr>
<tr>
<td>Ed Desmond</td>
<td><a href="mailto:Ed.Desmond@cdph.ca.gov">Ed.Desmond@cdph.ca.gov</a></td>
<td>510-412-3781</td>
<td>Chief of Mycobacteriology Technical info Special requests</td>
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<td>Technical info Special requests</td>
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<td>510-412-3949</td>
<td>General info</td>
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References

• PSQ for detection of XDR TB

• 2\textsuperscript{nd}-line CSDT by MGIT 960

• MDDR results & CDST by AP (CDC’ study)
Thank You!

Questions & Comments?