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.
  - **P**hone: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
- 1st 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 1st-line drugs.
  - Mutations detected, test 1st-line & 2nd-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!

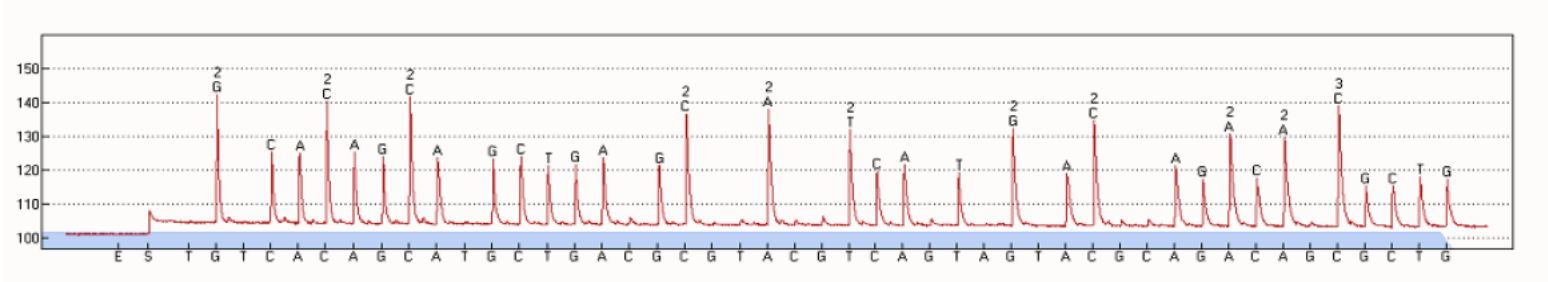
Pyrogram reveals NT sequences

Sample ID: **H37RV**

Well: E6
PSQ run: 05_24_13_ALL_SL
Entry ID: rB-S1-507-521-021413

Sequence library: MDL-8 target-rBs1[507-521]-rBs2A(522-533]-gAs2A-12-14-11-expanded-05-06-13 (2013-05-06, 7:30:59 PM)
Query sequence: GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTG

Result: RA01, rpoB1 no mutations within 507-521 **Score:** 100
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    1  GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTG 45
           |||||||||||||||||||||||||||||||||||||||||||||
Library  1  GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTG 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%)

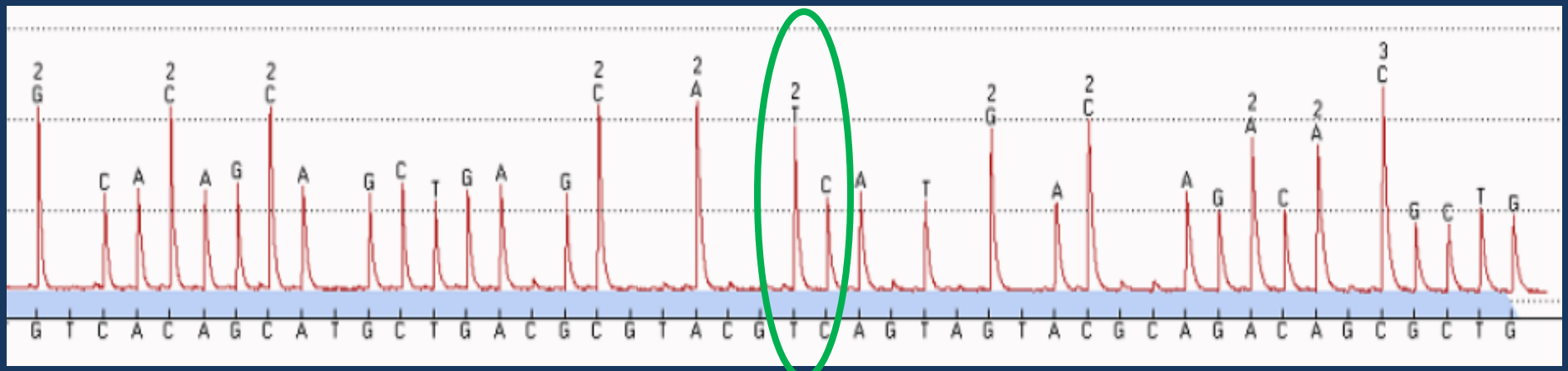
```
Query    1  GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCC~GCTG 45
           |||||||||||||||||||||||||||||||||||||||||| ||||
Library  1  GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCCGCTG 46
```

Hit 3: **RA01-02,rpoB1 no mutations within 507-521,(2C misread at 519)**

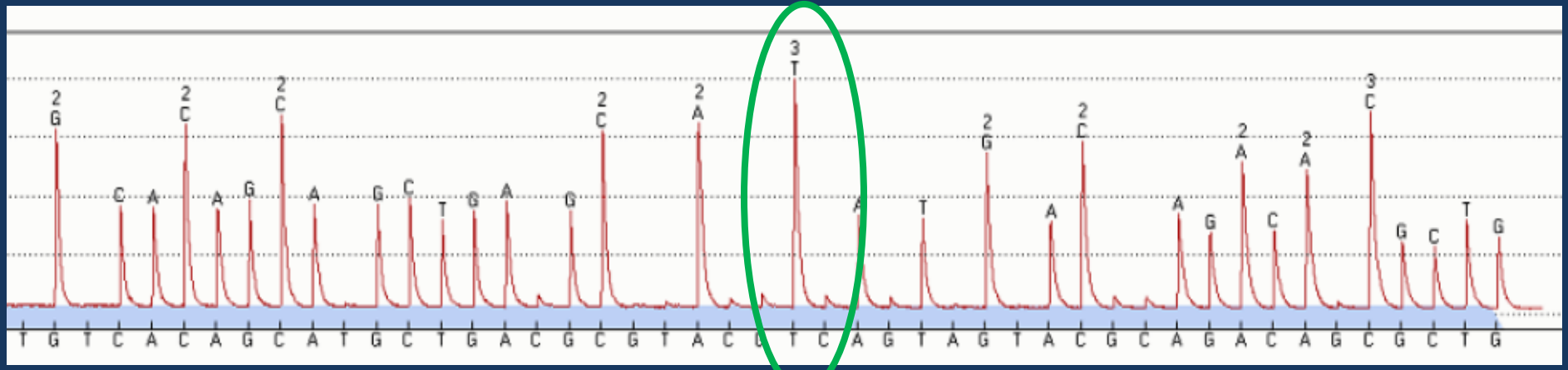
Score: 97.2
Identities: 44/45 (98%)
Gaps: 1/45 (2%)

```
Query    1  GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTG 45
           |||||||||||||||||||||||||||||||||||||||||| ||||
Library  1  GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACC~GCTG 44
```

Wildtype, *rpoB* 514 TTC



A silent mutation, *rpoB* 514 TTT



CDST

- Culture-based DST by MGIT 960
  - 1st-line: RIPE
  - 2nd-line: MACE, will add KAN after validated
- Reflexed 2nd-line DST
  - When R to any 1st-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

Name	Email account	Phone/Fax	Functions
TB Ref lab	CDPHTBDST@cdph.ca.gov	510-412-3949 (phone) 510-412-3704 (Fax)	Request for PSQ approval Notification of DST submission General info
Ed Desmond	Ed.Desmond@cdph.ca.gov	510-412-3781	Chief of Mycobacteriology Technical info Special requests
Grace Lin	Grace.lin@cdph.ca.gov	510-412-3929	Technical info Special requests
Steven Yu	Steven.Yu@cdph.ca.gov	510-412-3949	General info

References

- PSQ for detection of XDR TB
  - Lin SYG, et al. JCM 2014; 52:475-482.
- 2nd-line CSDT by MGIT 960
  - Lin SYG, et al. JCM 2009; 47:3630-3634.
- MDDR results & CDST by AP (CDC' study)
  - Campbell, et al. AAC 2011, 52:2032-2041.

Thank You!

Questions & Comments?