



Improved PZA Broth Susceptibility Testing on the VersaTREK

The Use of Tween

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Demystifying Pyrazinamide – Challenges and Opportunities
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VersaTREK

- Measures changes in the headspace pressure
- Middlebrook 7H9 Broth with Cellulose Sponges
 - Solid matrix
 - Surface area
- Detection & Recovery
 - All specimen sources
- Susceptibility Testing



What's the Problem?

False Resistance to PZA by broth methods

- Reported by both FDA approved broth platforms
 - Comparable platforms for DST
 - 98.5% Agreement between platforms
 - 97% Concordance for PZA testing (Espasa et al, JCM, 2012)
 - VersaTREK: >60% (1st hand experience)
 - MGIT: >40% False Resistance (Chedore et al, JCM, 2010)
- What's causing the false resistant results?
 - Variable drug stability in the acidic test medium
 - Difficulty preparing a uniform inoculum
 - Large inoculum impairs pyrazinimide activity

First Hand Experience Failed Proficiency Testing (PT)

- We were experiencing false PZA resistance
 - Multiple failed PT due to false resistance to PZA
 - Overcalling PZA resistance by >60%
- What can we do to lower the rate of false resistance and provide more accurate & reliable results?



Validation & Implementation of *pncA* Sequencing

- *pncA* - responsible for activation of the prodrug PZA and hence PZA activity
- Mutations in *pncA* gene correlate with *in vitro* PZA resistance
- Sequence a 700 bp product of the *pncA* gene and the upstream promoter region

Map of the *pncA* Gene with Nucleotide Polymorphisms and Amino Acid Changes

pncA (H37Rv) Polymorphism map

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1 M R A L I I V D V Q N D F C E G G S L A
1 ATGCGGGCGTTGATCATCGTGCAGCGTGCAGAAACGACTTCTGCGAGGGTGGCTCGCTGGCG
1 10 20 30 40 50
21 V T G G A A L A R A I S D Y L A E A A D
61 GTAACCGGTGGCGCCGCGTGGCCCGCCATCAGCGACTACCTGGCCGAAGCGGGGAC
61 70 80 90 100 110
41 Y H H V V A T K D F H I D P G D H F S G
121 TACCATCAGTCGTGGCAACCAAGGACTTCCACATCGACCCGGGTGACCACCTTCTCCGGC
121 130 140 150 160 170
61 T P D Y S S S W P P H C V S G T P G A D
181 ACACCGGACTATTCTCGTGGCCACCGCATTGCGTCAGCGGTACTCCCAGCGCGGAC
181 190 200 210 220 230
81 F H P S L D T S A I E A V F Y K G A Y T
241 TTCCATCCAGTCTGGACACGTCGGCAATCGAGGCGGTGTTCTACAAGGGTGCCTACACC
241 250 260 270 280 290
101 G A Y S G F E G V D E N G T P L L N W L
301 GGAGCGTACAGCGGCTTCAAGGAGTGCAGGAGAACGGCACGCCACTGCTGAATTGGCTG
301 310 320 330 340 350

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121 R Q R G V D E V D V V G I A T D H C V R
361 CGGCAACCGCGCGTTCGATGAGGTGATGTGGTCGGTATTGCCACCGATCATGTGTGGCG
361 370 380 390 400 410
141 Q T A E D A V R N G L A T R V L V D L T
421 CAGACGCGCGAGGACCGGGTACGCAATGGCTTGGCCACCAGGGTGTGGTGGACCTGACA
421 430 440 450 460 470
161 A G V S A D T T V A A L E E M R T A S V
481 GCGGGTGTGTGGCCGATACCACCGTCGCCGCGTGGAGGAGATGCGCACCGCCAGCGTC
481 490 500 510 520 530
181 E L V C S S *
541 GAGTTGGTTTGCAGCTCCTGA
541 550 560

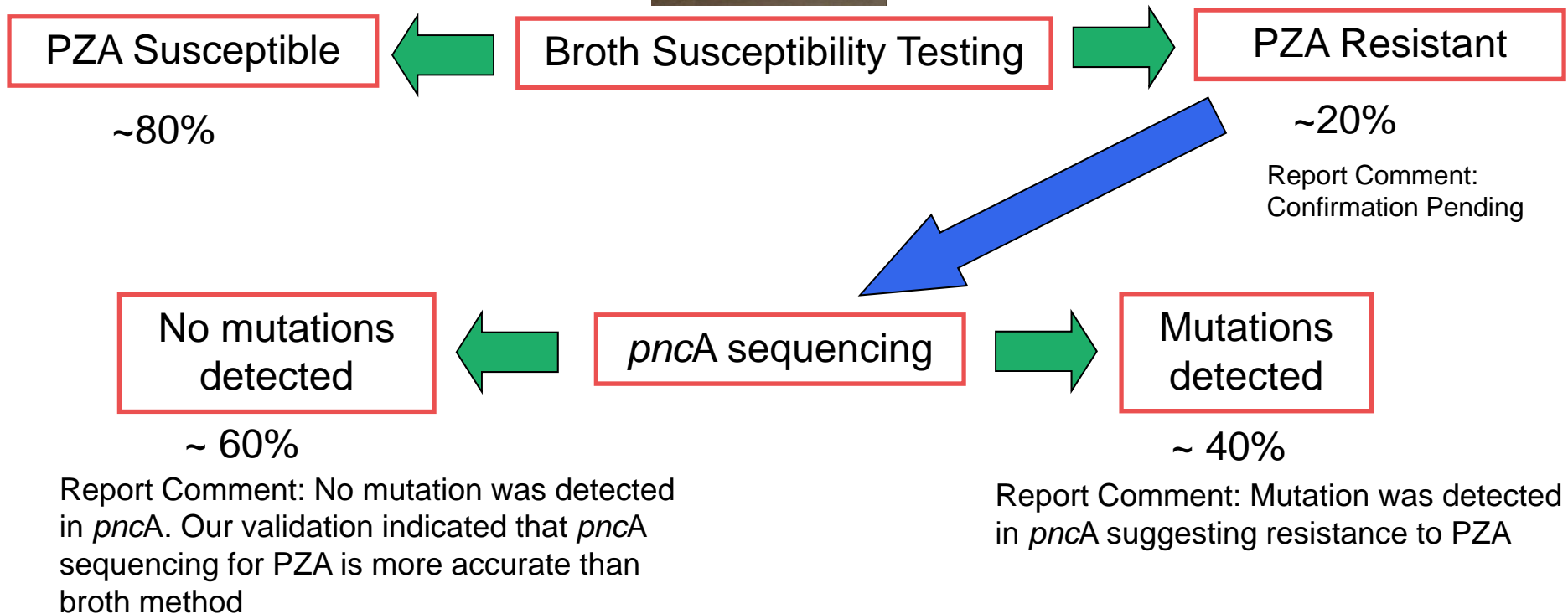
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Silent Coding change Insertion or Deletion (D)

Custom nucleotide library created during validation

Updated if new polymorphisms are identified that provide PZA resistance

Current PZA Susceptibility Testing Workflow



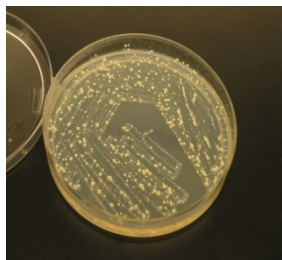
High Proportion being Reflexed to Sequencing

- Sequencing is a reliable alternative
 - Technically complex
 - Expensive
 - Limited to a few reference laboratories
- What can we do to reduce the amount of isolates being reflexed to sequencing?
 - Looks like an inoculum issue
 - Reinforced the use of a standardized inoculum in the lab but we were still having problems

Is Tween the Answer?

- Study:
 - Add the surfactant Tween to the inoculum seed bottle to see if it will reduce the occurrence of false resistant PZA results
- Hypothesis:
 - Tween reduces clumping to produce a more uniform and consistent inoculum

Study Design

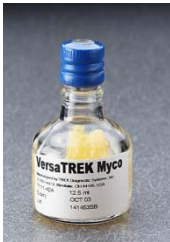


M. tuberculosis
culture isolate

Prepare
standard
inoculum



Seed bottle
with Tween



Seed bottle
without Tween

Incubate in
VersaTREK until
bottles signal;
use within 48-72hrs



2 control bottles
(no PZA)



2 test bottles
with PZA



2 control bottles
(no PZA)



2 test bottles
with PZA

Incubate in VersaTREK
instrument

Test bottle is considered
positive (resistant) if it signals
within 3 days of the control
bottle

Results- In Favor of Tween

Broth result	<i>pncA</i> sequencing results		Agreement (%)	# requiring confirmation of resistance*	Average Days to Positivity for Seed Bottle
	Susceptible	Resistant			
No Tween					
Susceptible	7	0	13/52 (25%)	45/52 (87%)	4.4
Resistant	39	6			
Tween added					
Susceptible	40	1	45/52 (87%)	11/52 (21%)	4.5
Resistant	6	5			

*all resistance is confirmed using another method or another laboratory

Conclusions

- Improved PZA broth susceptibility testing by increasing the agreement with *pncA* sequencing from **25%** of isolates without Tween to **87%** of isolates with Tween
- Significantly increases confidence in the broth testing results and reduces the number of isolates that need to be retested in order to confirm resistance by **66%**

Summary

- PZA susceptibility testing using a broth method is time-consuming and false resistance is common.
- Confirmation of resistance is expensive and time-consuming and false resistance adds to that burden.
- *pncA* sequencing is an excellent alternative to broth methods but it is not available in all clinical mycobacteriology laboratories
- Addition of Tween to the seed bottle provides a more consistent inoculum that reduces false resistance and reduces the number of isolates requiring repeat testing to confirm resistance.

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