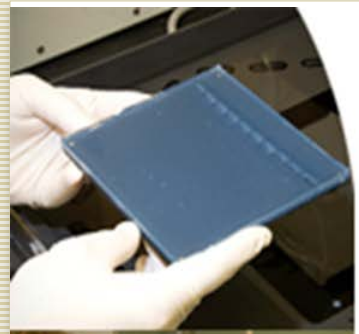
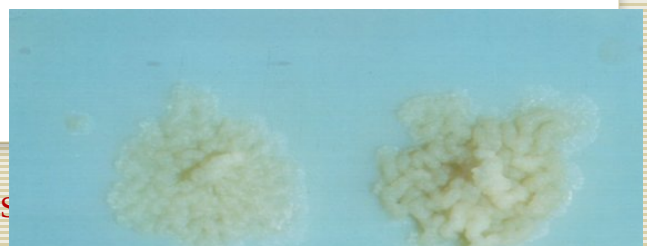


# *Issues in Tuberculosis Drug Susceptibility Testing: TB Subcommittee White Papers*

Dave Warshauer, PhD D(ABMM)
Deputy Director, CDD
Wisconsin State Laboratory of Hygiene



# APHL TB Subcommittee

- John Bernardo
- Ed Desmond
- Ann Gaynor
- Frances Jamieson
- Ken Jost
- Bev Metchock
- Will Murtaugh
- Bill Slanta
- Angela Starks
- Becky Temple
- Dave Warshauer

# Drugs being Addressed

- Pyrazinamide
- Rifamycins
  - Rifampin
  - Rifabutin
- Ethambutol
- Fluoroquinolones
  - Moxifloxacin
  - Levofloxacin
  - Ofloxacin

# What's a "White Paper"

"A **white paper** is an authoritative report or guide informing in a concise manner about a complex issue and presenting the issuing body's philosophy on the matter. It is meant to help readers understand an issue, solve a problem, or make a decision."

Courtesy of Wikipedia

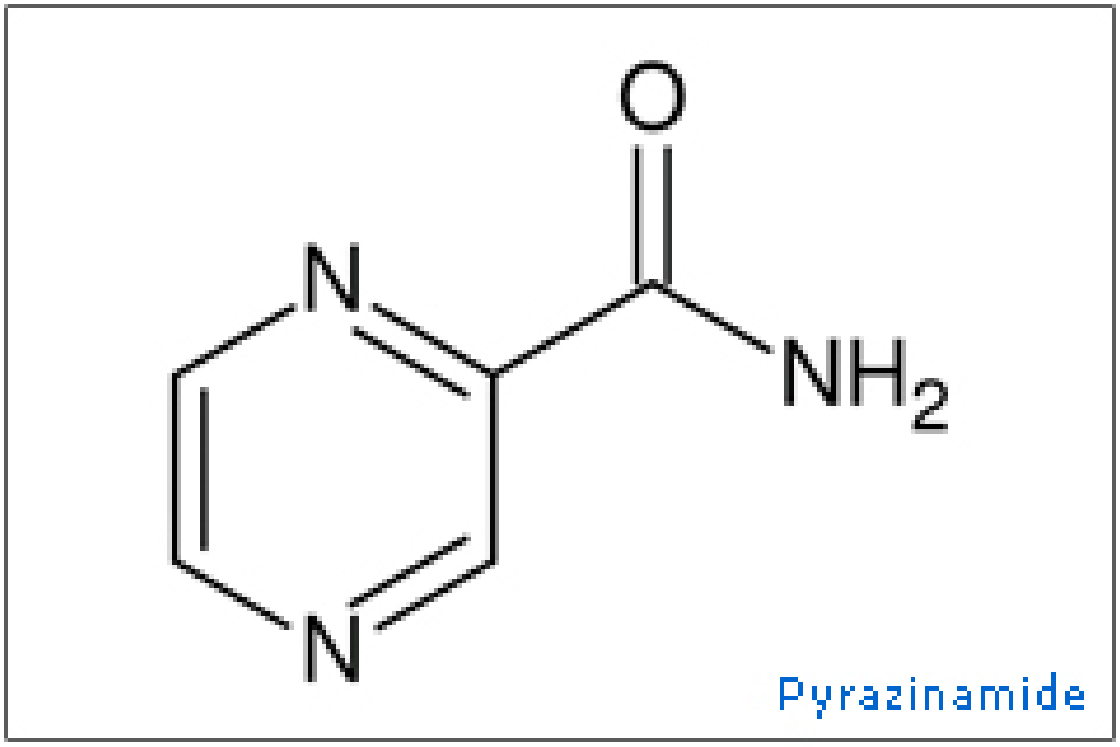
# Organization of the White Papers

- Background
  - Role in treatment regimen
  - Mechanism of action
  - Mechanisms of resistance
  - Drug side-effects
- Practical laboratory issues
  - Culture-based DST
  - Molecular methods
  - Other tests for resistance detection
  - Proficiency Testing

# Organization of the White Papers

- Impact on clinical outcomes
  - Prolonged therapy
  - Use of more toxic drugs
  - Creation of MDR-TB
  - Treatment failure
- Areas of ongoing research
  - Defining role of new mutations in resistance
  - New resistance determinants
- Testing guidance
- References

# PZA White Paper

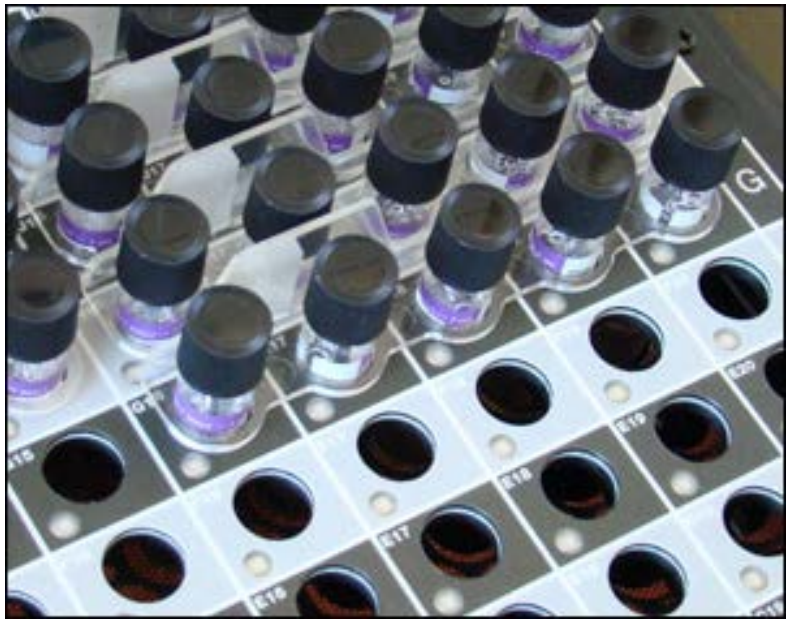


# PZA

- Critical component of first-line drug combination therapy
- Shortens chemotherapy regimen to 6 months (WHO Treatment Guidelines 4th Ed)
- Significant effect against non-replicating "persister" organisms or slowly replicating bacilli at acid pH (5.5)
  - Kills bacilli not eliminated by other TB drugs

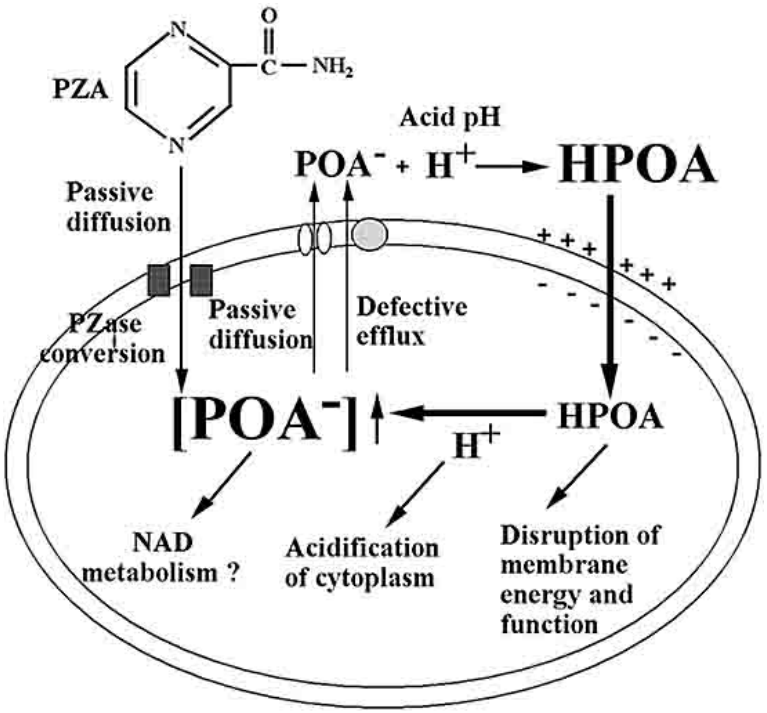
# PZA

- Inactive against organisms in the growth phase during culture conditions at neutral pH
  - Use pH 6.8 for culture-based DST



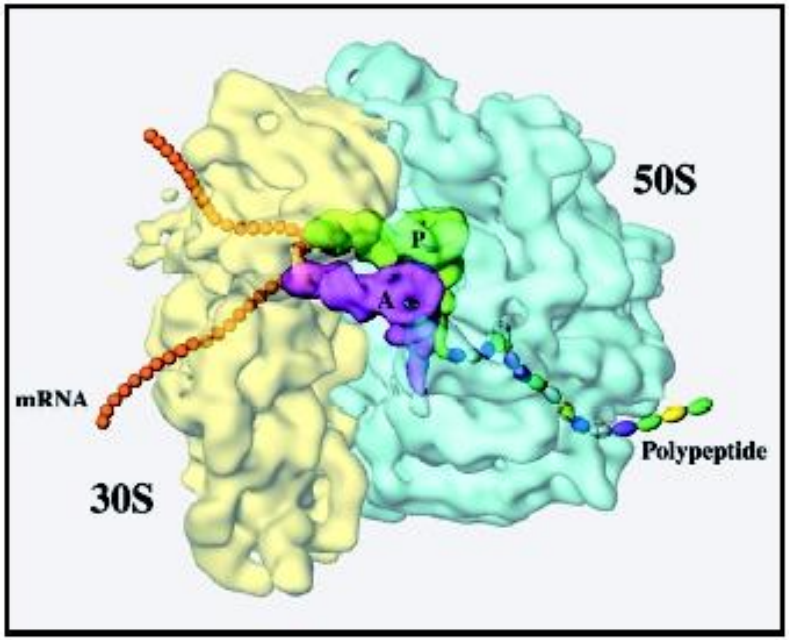
# Mechanism of Action

- Prodrug that requires conversion to active form, pyrazinoic acid (POA)
- Converted by pyrizinamidase (Pzase) encoded by *pncA* gene
- POA expelled by putative efflux pump
- Outside of cell POA protonated and re-enters
- H+ ⟶ acidification of cytoplasm



# Mechanism of Action

- POA also targets ribosomal protein S1 (RpsA)
  - Inhibits protein synthesis (Shi et al, Science 2011)




http://www.biologyreference.com/images/biol_04_img0400.jpg

# Mechanism of Resistance

- Primary Mechanism—Loss of PZase activity
  - Due to mutations in *pncA* gene
  - Affect catalytic sites and $Fe^{2+}$ binding site
    - PZA not converted to POA
- Mutations found in 72-97% of PZA resistant isolates
  - Widely distributed throughout the gene

# Mechanism of Resistance

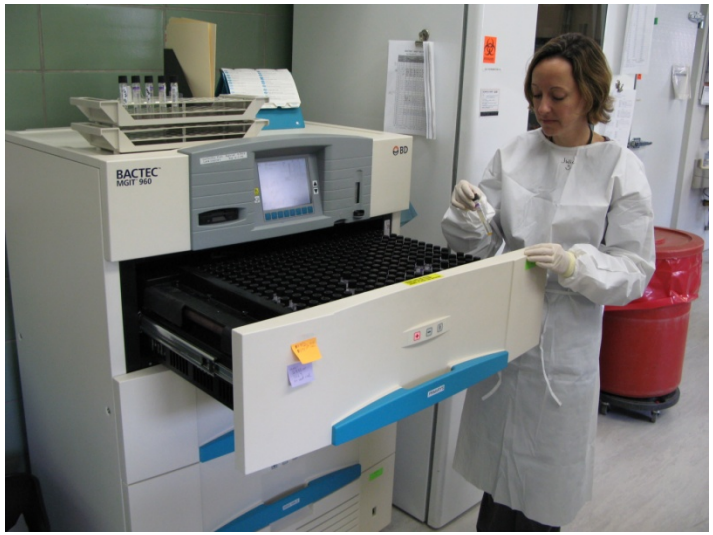
- All *pncA* mutations do not result in resistance
  - Newly recognized mutations require evidence to determine if they truly cause phenotypic resistance.
  - Some PZA R isolates don't have any *pncA* mutations
- Other mechanisms of resistance
  - Efflux of POA
  - Altered PZA uptake
  - Impaired POA binding to the drug target
  - Lack of pncA expression

# Practical Laboratory Issues

# Susceptibility Testing of M.tb

- Agar Proportion Method
- Bactec 460 Radiometric Method
  - Discontiued in 2011
- **MGIT**
- Versa Trek
- Trek Microbroth dilution MIC
  - Not FDA approved
  - PZA not included

**Our MGIT**



# Other Non-FDA Approved Methods

- Resazurin microtitre assay (REMA)
  - Detect growth in microtiter well format using redox reactions
- Colorometric nitrate reductase assay
- Dimethylthiazol diphenyl tetrazolium bromide (MTT) redox reaction
- Nitrate reductase assay

# PZA Deaminase Test

- Detects deaminization of PZA to POA and ammonia
  - pink color in the medium due to pH change
- Negative test correlates well with resistance
- Cannot interpret a positive test as susceptible
  - May be resistant by other mechanisms

# Reproducibility Issues

- pH effects
  - PZA active only in acid environment
    - Commercial systems use pH 6.8
    - A large inoculum 10^7 to 10^8 increases pH leading to false resistance
    - 10^6/ml leads to a small increase in pH
  - Too low inoculum or old cultures may lead to false susceptibility

# False Resistant PZA DST Results

| | No. reported as Resistant/total reports (% reported as Resistant) | | |
|---|---|---|---|
| **Isolate*** | Bactec 460 | MGIT | VersaTREK |
| **A** | 0/17 (0) | 1/64 (2) | 0/5 (0) |
| **B** | 0/17 (0) | 7/62 (11) | 0/3 (0) |
| **C** | 0/17 (0) | 20/62 (32) | 3/3 (100) |
| **D** | 0/17 (0) | 21/63(33) | 3/3 (100) |
| **E** | 0/17 (0) | 0/64 (0) | (0) |

*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

Data from 2010 CDC Model Performance and Evaluation Program for MTBC DST
Chedore, P., et al., 2010. J. Clin. Microbiol. 48:300-301.

# Proficiency Programs

- CAP is the only CLIA-approved PT program for DST
  - Cannot ship resistant strains
  - Use the same strain every challenge
- WHO proficiency test panels
  - Supranational Reference Laboratories
  - PZA not included

# Impact on Clinical Outcomes

# Clinical Outcome

- PZA is a critical component of first-line drug combination therapy
  - For both pan-susceptible and MDR TB
  - Used in the first 2 months of therapy
- If PZA is reported as resistant the length of therapy is increased by three months
  - False-resistance may result in prolonged, unnecessary treatment

# Areas of Ongoing Research

http://www.peerresources.org/curriculum/lesson-plan-2-why-use-research/

# Mutations

- *pncA* gene mutations spread along entire gene as well as the upstream regulatory region for approx 700 bp
  - Large number of mutations published, but no predominant mutations
  - Some always associated with resistance, but ongoing studies needed to determine phenotypes of other mutations

# Other Areas of Research

- Critical concentration revisions
  - Research on development of new testing breakpoints is ongoing
  - MGIT currently 100 ug/ml
  - Trek 300 ug/ml
  - Likely not to change for FDA-approved tests
- Role of other gene targets in resistance?
  - *rpsA* gene—Altered POA binding to ribosomal protein S1
  - *panD*—involved in co-enzyme A synthesis

# Guidance

# Laboratory Considerations to Optimize Results

- Use fresh cultures for preparation of inocula for culture-based DST
- Ensure standard inoculum
- Consider using a lower inoculum
  - MGIT---use Day 3-5 seed vial
  - Vortex well and allow to settle for 20 min
  - Take aliquot from top and dilute 1:5
- Repeat DST if initially resistant
  - Perform *pncA* sequencing

# Laboratory Considerations to Optimize Results

- If using Pzase, a negative result correlates well with resistance, but not all positive isolates can be considered susceptible.
- All mono-resistant PZA isolates should be identified to determine if *M. bovis* or *M. bovis* BCG
  - Intrinsically R to PZA

# Thank You