Is PZA susceptibility testing a direct path to the exploding head zone?

Dr. Desmond has no conflicts to disclose.

The recall

On 8/1/2024, the CDC Tuberculosis Reference Laboratory received a notification from Becton, Dickinson and Company (BD), the manufacturer of the BD BACTEC MGIT 960 PZA Kit, that multiple lots of this reagent kit, distributed in 2023 and 2024, should be immediately discarded by the customers because they may intermittently produce falsely resistant results for pyrazinamide (PZA) during susceptibility testing of *Mycobacterium tuberculosis* (MTB) isolates.

The other reported issue

PZA –containing MGIT tubes which gave a susceptible result (i.e. didn't trigger the growth indicator) sometimes exhibited flaky visible growth at the bottom of the tube.

How common is PZA resistance?

Wang, et al. 2023 J Chemother 35(7): 583

- Overall worldwide 29%
- Americas 17%
- MDRTB cases in the Americas 51%

PZA and the exploding head zone

- PZA is different
- Phenotypic testing requires use of an undefined biological component which is inherently variable
- Sequencing is more reproducible and likely more reliable than phenotypic testing
- Inferring PZA susceptibility /resistance from whole genome sequencing takes too long
- Note: APHL published a “white paper” on PZA problems in 2016: https://www.aphl.org/aboutAPHL/publications/Documents/ID-PZA_WhitePaper_0216.pdf

PZA is different (and important)

- How it was discovered: animal testing of nicotinamide analogs
- Give for two months only
  - May be too toxic for some fragile patients & too toxic to give longer
  - Appears to work early, even if lesions are big
- Enables shortening of treatment regimens (**a BIG +**)
- Works in an acidic environment (all types of lesions, targets inactive bacilli)
  - Susceptibility testing has to be done in an acidified environment ☹
- It's a prodrug (like INH)
  - Needs to be activated by pyrazinamidase (coded for by *pncA* gene)
  - Active pyrazinamidase is not essential for the survival of TB bacilli
- Accuracy of culture-based DST appears to be very inoculum-dependent ☹

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Quality Control of Individual Components Used in Middlebrook 7H10 Medium for Mycobacterial Susceptibility Testing

LINDA S. GUTHERTZ, MARGUERITE E. GRIFFITH,* ELEANOR G. FORD, J. MICHAEL JANDA, AND THADDEUS F. MIDURA

Microbial Diseases Laboratory, California Department of Health Services, 2151 Berkeley Way, Berkeley, California 94704

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The acceptability of different lots of commercial components which constitute our basal medium for susceptibility testing of mycobacteria was evaluated. The basal medium consisted of Middlebrook 7H10 agar supplemented with 10% oleic acid-albumin-dextrose-catalase and 0.5% glycerol. Studies were performed by using three separate microbiologic assays, and results were compared with parallel tests on previously standardized and acceptable lots of media. Components were rejected if comparison with standardized medium showed a major change in growth support or susceptibility status of any reference strain to any antimicrobial agent tested. Of the components tested in such a manner, 7 of 23 (30%) lots of 10% oleic acid-albumin-dextrose-catalase, 2 of 13 (15%) lots of Middlebrook 7H10 agar, and 0 of 5 lots of glycerol were found to be unacceptable. This study demonstrates that individual lots of components of this basal medium may vary significantly in their suitability for susceptibility testing, and failure to detect such variation may dramatically affect susceptibility profiles.

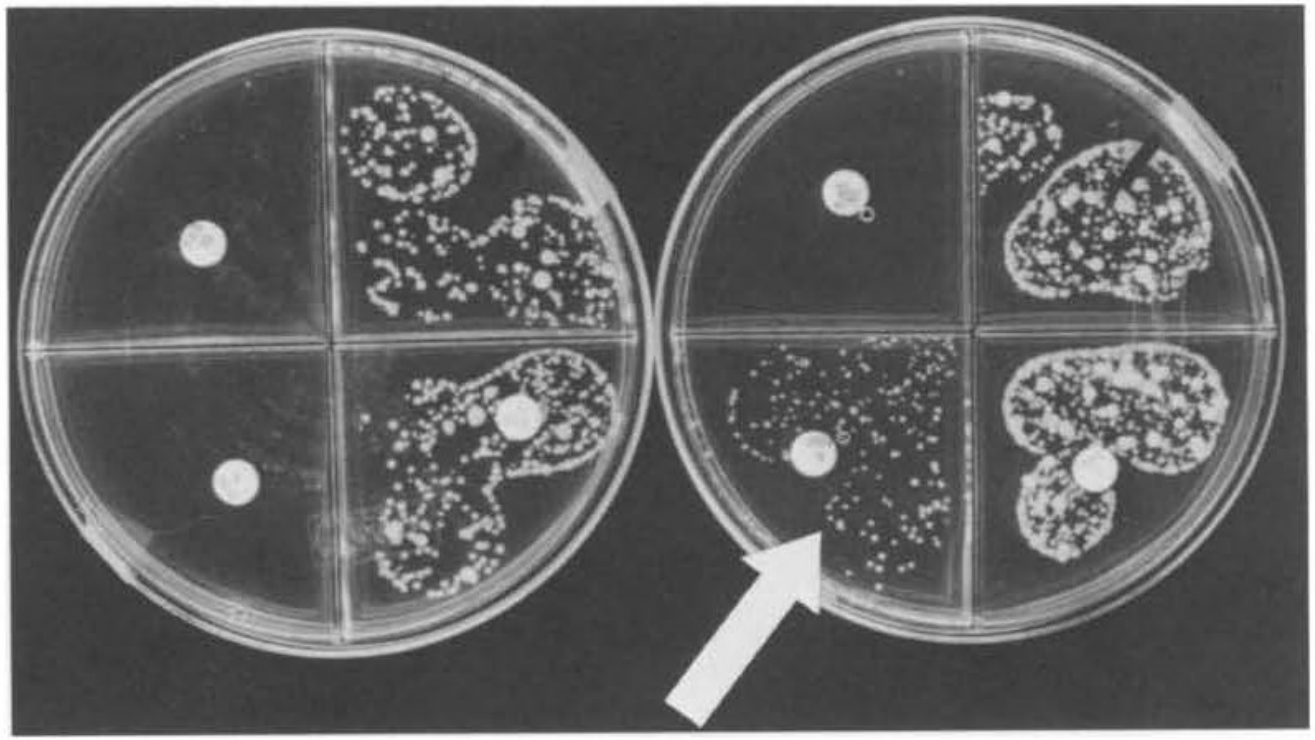


FIG. 2. Drug binding by a new component and the resulting false resistance to drug apparent in the standard concentration assay. The standardized medium on the left contains a quadrant without drug and three quadrants with drug supplied by disks. The test medium on the right was prepared identically to the standardized medium and in parallel, except that a new lot of OADC was substituted. All quadrants were inoculated with 0.1 ml of a suspension of *Mycobacterium scrofulaceum*. The arrow indicates the quadrant in which drug binding by OADC permitted growth of drug-susceptible organisms.

New batch of OADC can impact drug activity

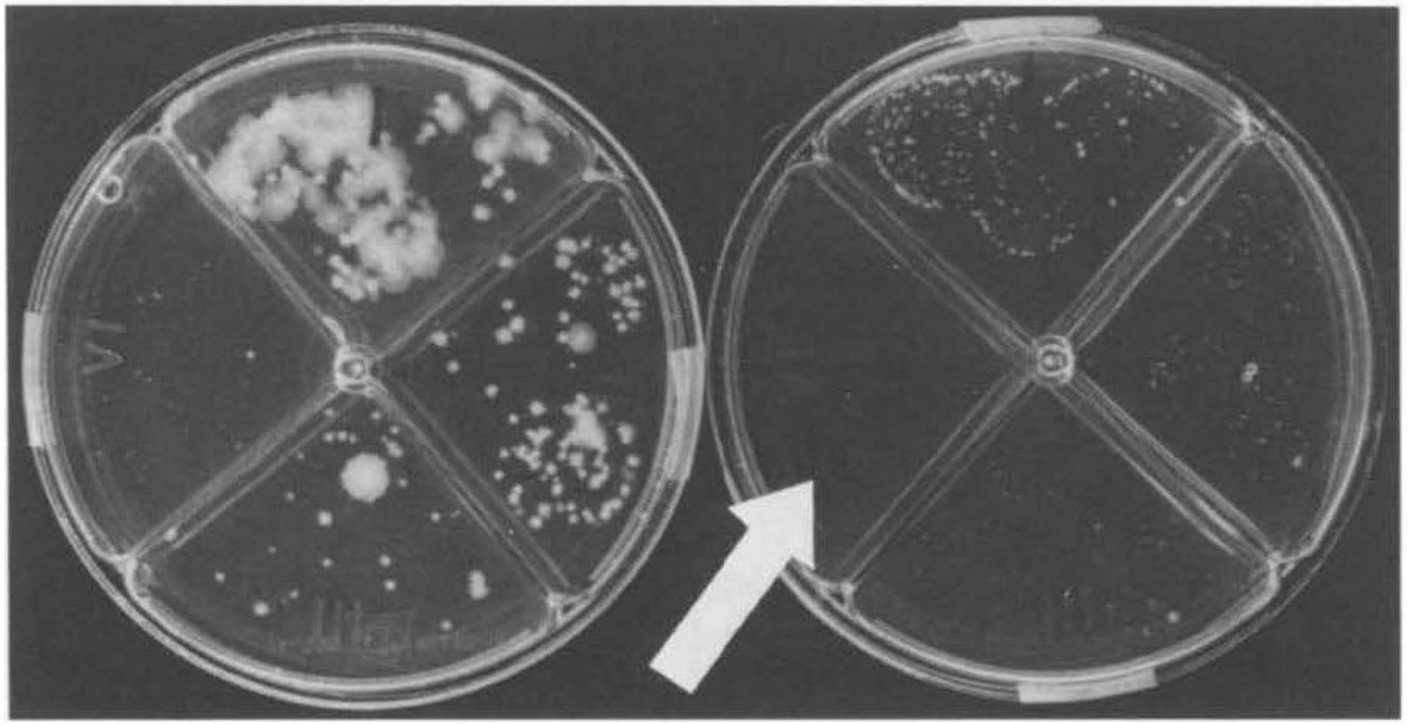


FIG. 1. Reduction of colony size by a new component and the resulting false susceptibility to drug apparent in the comparative resistance assay. The standardized medium on the left contains a quadrant without drug and three quadrants with increasing concentrations of ethionamide. The test medium on the right was prepared identically to the standardized medium and in parallel, except that a new lot of OADC was substituted. All quadrants were inoculated with 0.1 ml of a suspension of *M. tuberculosis*. The arrow indicates the quadrant in which colony size was reduced beyond visibility.

New batch of OADC can impact growth support

QC testing to minimize impact of changes in OADC composition

Past California lab practice:

- Ask OADC manufacturer to set aside an entire production batch of OADC and send two vials to CA lab
- Test vials for growth support and drug activity *with all drugs* and *including MIC testing of a control strain.*
- If acceptable, purchase entire lot

Problems with this approach:

- A lot of work and time-consuming
- What did the manufacturer do with the remainder of a batch if we rejected it? [Answer: sell it to somebody else]

What is BD doing to prevent problems such as the one that resulted in the PZA test medium recall?

- BD has a quality control team who promote acquisition and incorporation of consistent reliable ingredients for PZA test medium
- BD has a TB strain with slightly elevated MIC to PZA, as an indicator in case a new batch of the medium has a tendency to yield false resistant results.
- Despite these precautions, will future batches of PZA test medium occasionally lead to increased (and unreliable) resistance to PZA?

Testing MGIT MIC as a way of detecting lot to lot variation

- The BD PZA reagent quality issue was detected early by the California Department of Public Health because of abnormally high MICs for H37Rv, with an increased frequency of likely false-resistant phenotypic results for clinical strains.
- The H37RV MIC for PZA is less than the usual test concentration for this drug, necessitating the development of a specialized MIC protocol (which the CA lab is willing to share).
- It is possible, but labor-intensive, to test MICs for each drug to be tested whenever a new batch of growth medium or OADC is received.
- Not necessary for every lab to do so, but those who can should take on the MIC testing for PZA for new batches of OADC. BD is not likely to take this on.

Issues in *Mycobacterium tuberculosis* Complex (MTBC) Drug Susceptibility Testing: Pyrazinamide (PZA)

BACKGROUND

Pyrazinamide (PZA) is a critical component of first-line drug combination therapy for Mycobacterium tuberculosis complex (MTBC) including both susceptible and multi-drug resistant tuberculosis (MDR TB). Inclusion of PZA has shortened the previous 9–12 month chemotherapy regimen to 6 months.[1] PZA has also become an essential part of MDR TB treatment regimens that include novel compounds now clinically available, such as bedaquiline.[2,3] PZA is inactive

Approaches to Improving Reproducibility and Specificity Using the MGIT 960 PZA DST Inoculum:

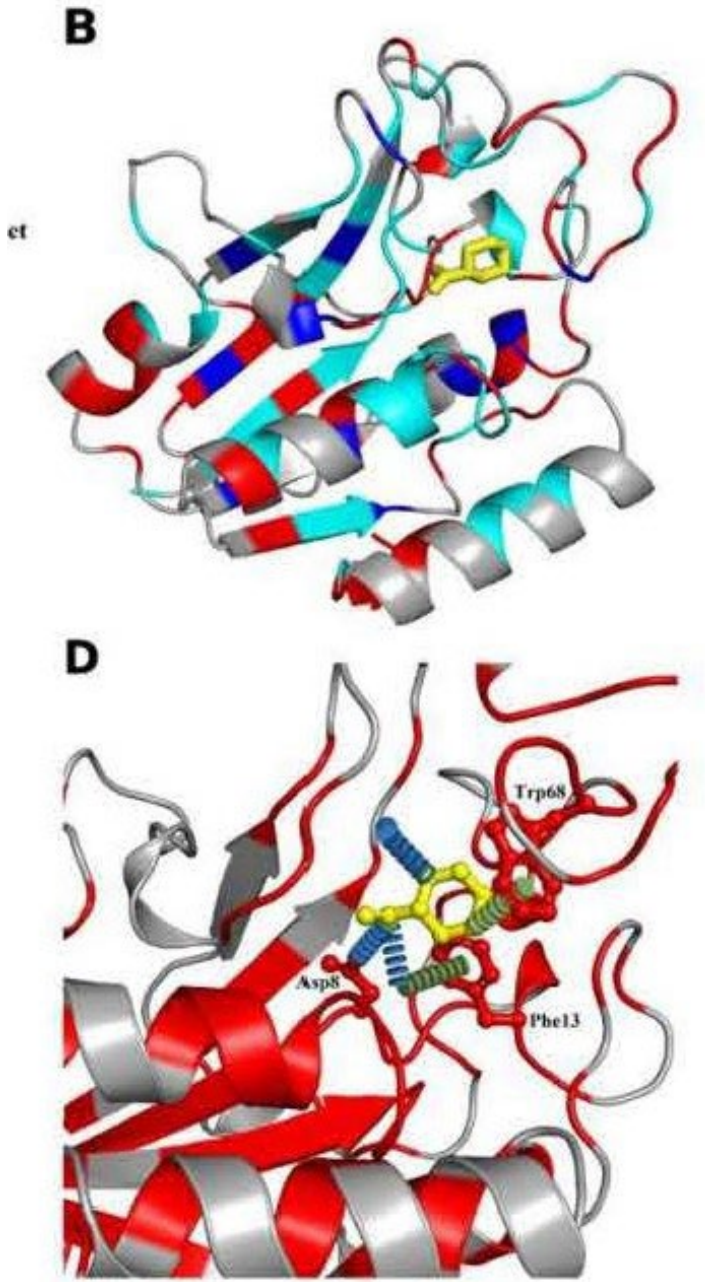
Lack of standardization of test inoculum significantly affects results. The manufacturer's recommended method for testing using MGIT 960 likely results in differences in the amount of organism used in testing. The concentration of MTBC in the MGIT 960 compared to the Bactec 460 is greater than 2.5 times, and there is variation in the concentration of the inoculum used in the MGIT 960, depending on the day of test set-up. If a much larger inoculum or non-homogenous inoculum (clumps) is used for testing, there is a high potential for false resistance.40,52,60 In order to ensure a comparable, uniform inoculum for each test, it has been suggested that the inoculum preparation be "standardized" to approximately 10^6 CFU/mL. This can be achieved by allowing the test inoculum to settle after removal of culture material from a primary MGIT 960 tube, removing supernatant and then diluting to a 0.5 McFarland standard.

Reference: Lin S-YG, Desmond E, Bonato D, Gross W, Siddiqi S. Multicenter Evaluation of Bactec MGIT 960 System for Second-Line Drug Susceptibility Testing of Mycobacterium tuberculosis Complex. J Clin Microbiol. 2009 Nov 1;47(11):3630–4.

What about the flakes of growth in the bottom of the PZA test broth?

- Growth indicator is not triggered
- If flakes of growth are pipetted out of the bottom of the tube and cultured in MGIT medium without drug, *M. tb* will grow.
  - Creates some discomfort in calling this susceptible.

- But what if you sequence the *pncA* gene and promoter from this growth and find that the wild type (susceptible) sequence is present?
  - Would that make you more comfortable in ignoring flaky growth that doesn't trigger the growth indicator?



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Structure guided prediction of Pyrazinamide resistance mutations in pncA

Malancha Karmakar[1,2,3], Carlos H. M. Rodrigues[1,2], Kristy Horan[4], Justin T. Denholm[3] & David B. Ascher[1,2,5*]

› Int J Antimicrob Agents. 2024 Apr;63(4):107053. doi: 10.1016/j.ijantimicag.2023.107053. Epub 2023 Dec 9.

Emerging insight of whole genome sequencing coupled with protein structure prediction into the pyrazinamide-resistance signature of Mycobacterium tuberculosis

Chun-Kai Huang [1], Ming-Chih Yu [2], Ching-Sheng Hung [3], Jung-Chun Lin [4]

Affiliations + expand

PMID: 38081550 DOI: 10.1016/j.ijantimicag.2023.107053

Basing susceptibility/ resistance calls on sequence and 3D modeling

- New *pncA*/promoter mutations associated with resistance continue to be found.
- 3D modeling can help predict whether the mutation could be expected to be associated with PZA resistance.
- Some level of phenotypic confirmation will enable confidence in the sequencing/modeling prediction.
  - How many phenotypic results should be required?
  - Note that some point mutations away from the active site of the enzyme might not cause resistance?

Thank you for your attention