



# A Less Expensive Approach to Prepare Seed Cultures for PZA Susceptibility Testing

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## INTRODUCTION

Pyrazinamide (PZA) is one of the first line drugs used to treat tuberculosis. Currently, there are two FDA-cleared platforms for PZA susceptibility testing of *M. tuberculosis* complex (MTBC): the BACTEC™ MGIT™ (Becton Dickinson) system (Fig. 1) and the Versa-TREK MYCO TB™ (TREK Diagnostic) system. The Texas Department of State Health Services Laboratory (DSHS Lab) uses the MGIT system which is the more commonly used method in the U.S.<sup>1</sup> Due to the cost, relatively short shelf-life, and purchasing obstacles associated with commercial Middlebrook 7H9 broth, the DSHS Lab proposed a modification to the current package insert protocol by replacing Middlebrook 7H9 broth or MGIT broth with sterile saline made in-house.<sup>2,3</sup> Only the PZA procedure was evaluated because all other drugs are tested by the indirect agar proportion method at the DSHS Lab<sup>4</sup>

## METHODS

The scope of this study included 25 randomly chosen MTBC positive isolates from April-July 2020. (Fig. 2) Following the PZA kit package insert, 0.1ml of a positive MGIT culture was inoculated into 10.0ml of 7H9 broth. 0.5ml of this mixture was then inoculated into a 7ml MGIT tube containing 0.8ml of growth supplement to make the seed MGIT. These same cultures were then set up using 0.85% saline dilution blanks instead of 7H9 broth.

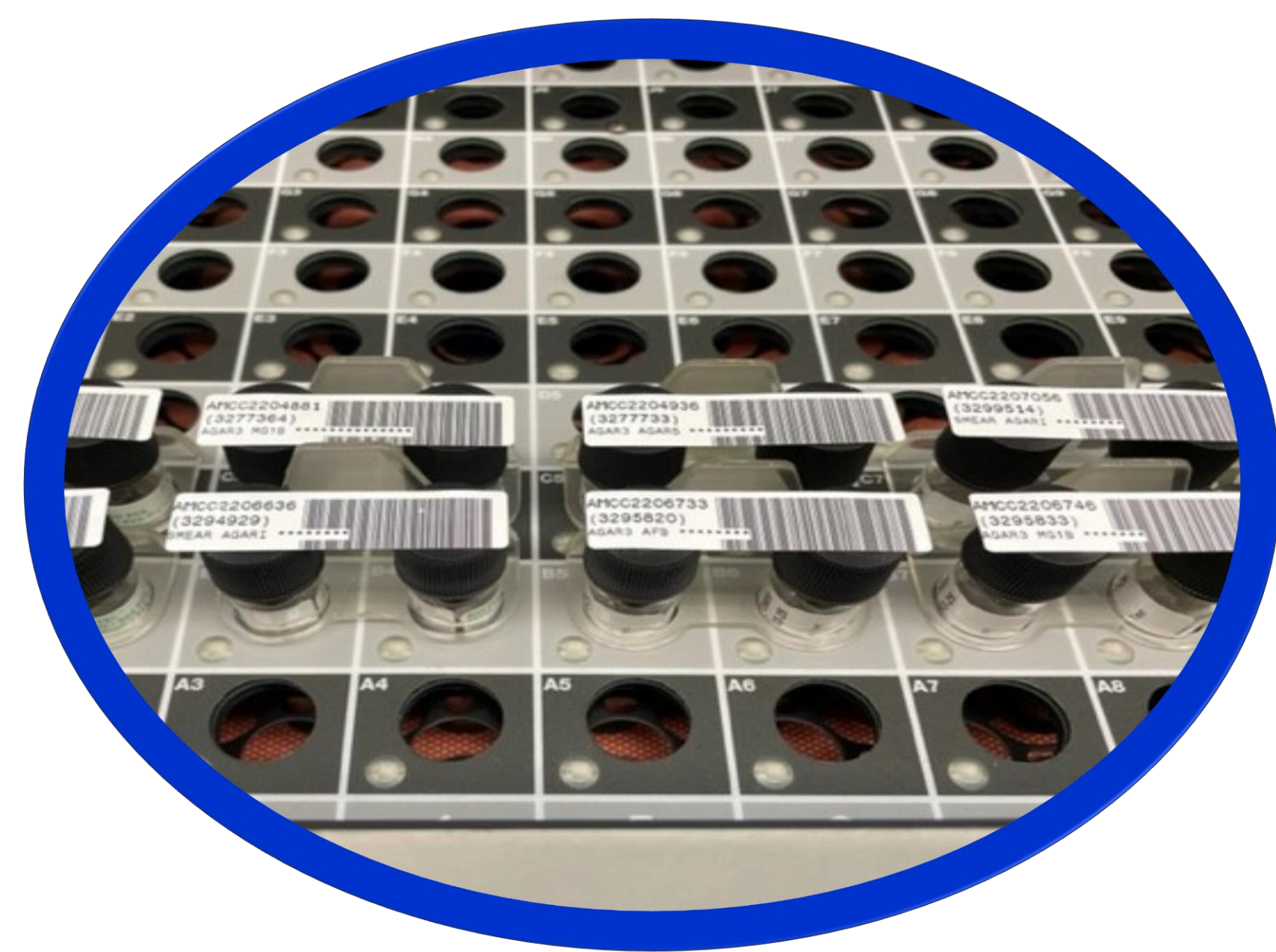


Figure 1: PZA tests in BACTEC MGIT 960 instrument

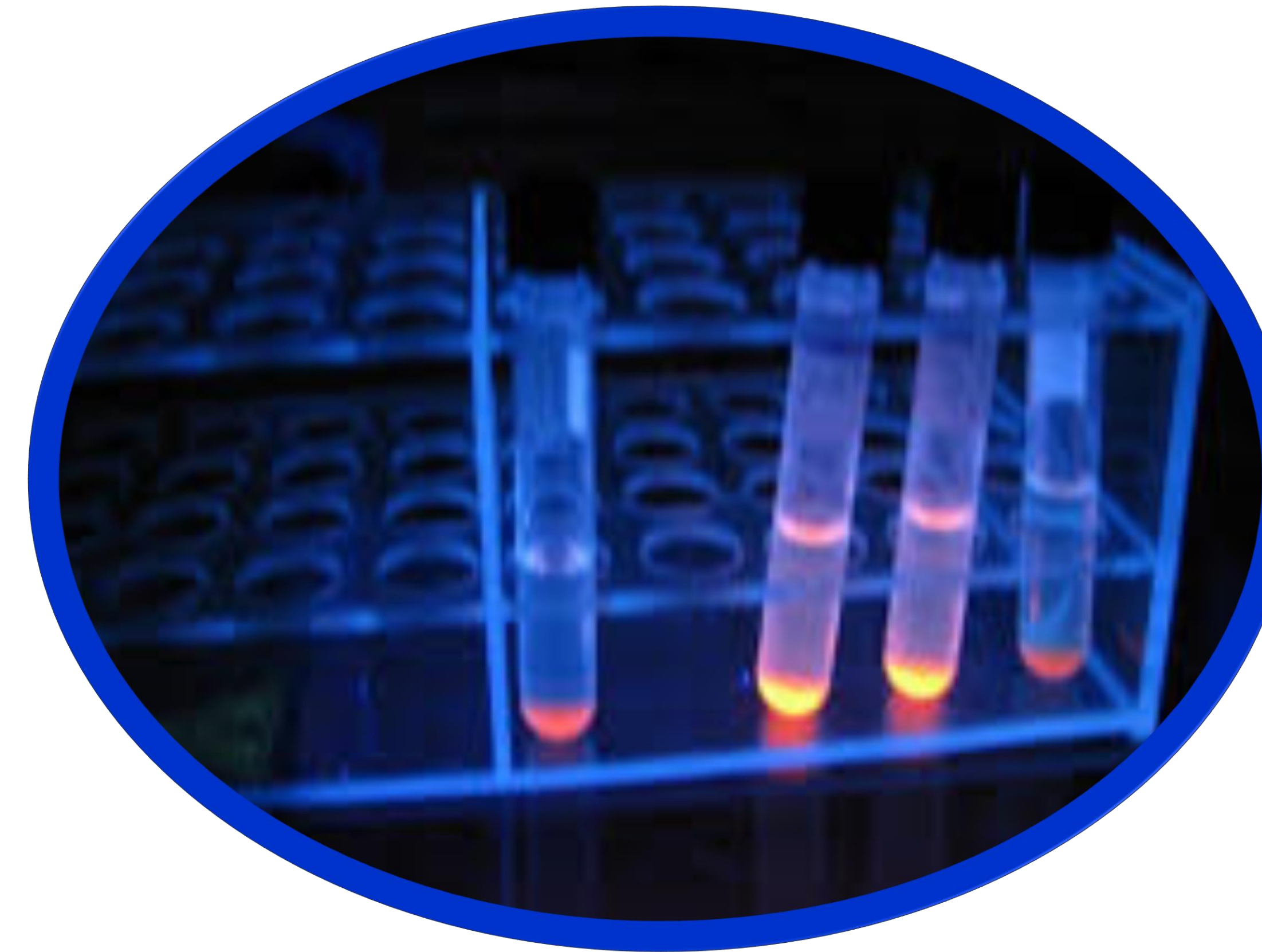


Figure 2 – Positive MGIT cultures used to make PZA seeds

## RESULTS

Our Team found that using 0.85% saline dilution blanks in place of 7H9 broth for the preparation of a seed MGIT yielded 100% categorical agreement in results.

★ 100% Overall Accuracy ★

20 isolates that tested Susceptible to PZA using 7H9 broth per package insert also tested Susceptible to PZA when using 0.85% saline for seed preparation. This same accuracy was observed when testing five Resistant isolates with both 7H9 broth and saline dilutions.

★ 100% Overall Precision ★

One of the Resistant isolates and two of the Susceptible isolates were tested by two different microbiologists on three different days. PZA results were repeatedly consistent using 7H9 broth and 0.85% saline for seed preparation.

In this section of the study, four seed MGITs (two seeds prepared with 7H9 broth, and two seeds prepared with saline) were repeated due to low/slow growth. This event is not an uncommon occurrence with this test and is not unique to either diluent.

## CONCLUSIONS

This study demonstrated that seed MGITs made with sterile saline performed just as well as seeds made with Middlebrook 7H9 broth, resulting in 100% sensitivity and specificity for 25 randomly chosen MTBC positive isolates. The phenomenon of organism clumping in solution was not observed to be any more or less frequent when using saline.

Our cost of a 10ml tube of 0.85% sterile saline made in-house is just \$0.17, as compared to \$5.05 per tube of commercially produced 7H9 broth and \$6.43 per MGIT tube (some distributors are charging as much as \$18.18 for a MGIT tube).<sup>5</sup> This saved our agency approximately \$4227.00 in 2020 alone. (Fig 3)

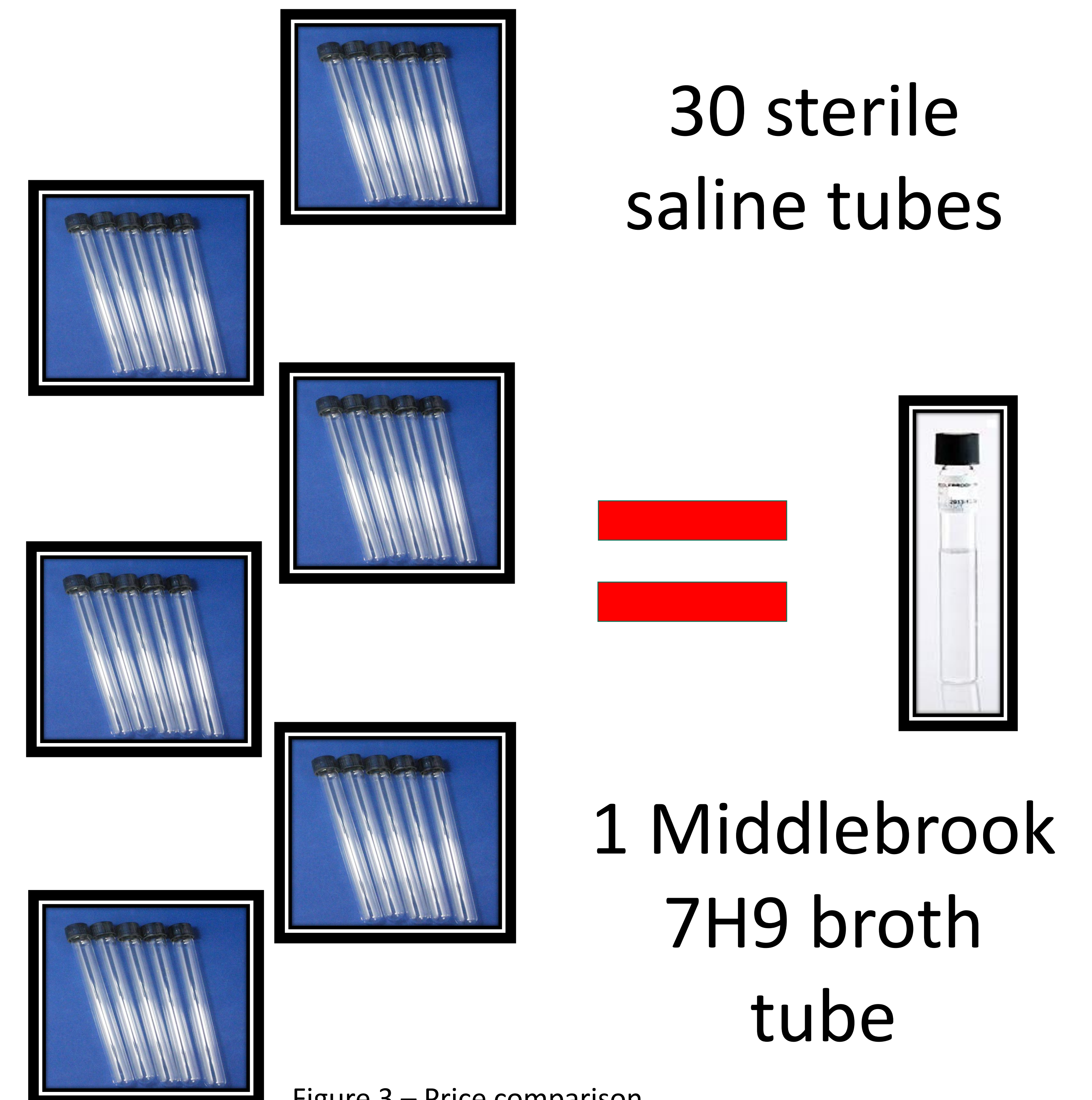


Figure 3 – Price comparison

1. Glenn P. Morlock, Frances C. Tyrrell, Dorothy Baynam, Vincent E. Escuyer, Nicole Green, Youngmi Kim, Patricia A. Longley-Olson, Nicole Parrish, Courtney Pennington, Desmond Tan, Brett Austin, James E. Posey, "Using Reduced Inoculum Densities of *Mycobacterium tuberculosis* in MGIT Pyrazinamide Susceptibility Testing to Prevent False-Resistant Results and Improve Accuracy: A Multicenter Evaluation", *Tuberculosis Research and Treatment*, vol. 2017, Article ID 3748163, 9 pages, 2017.  
2. BD BACTEC™ MGIT™ 960 PZA Kit for the Antimycobacterial Susceptibility Testing of *Mycobacterium tuberculosis* package insert. L-005486JAA(05). 2016-10  
3. Microbiological Branch 142 TB Reference Drug Susceptibility Testing of *Mycobacterium tuberculosis* complex by the BACTEC MGIT (Mycobacteria Growth Indicator Tube) 960 System  
4. Microbiological Branch 153 TB Reference Agar Proportion Indirect Susceptibility Test for *Mycobacterium tuberculosis* complex  
5. VWR.com online catalog