BD BACTEC™ MGIT™ 960 PZA Kit

For the Antimycobacterial Susceptibility Testing of *Mycobacterium tuberculosis*

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INTENDED USE

The BD BACTEC™ MGIT™ 960 PZA Kit is a rapid qualitative procedure for susceptibility testing of *Mycobacterium tuberculosis*, from culture, to pyrazinamide (PZA). The BD BACTEC™ MGIT™ 960 PZA Kit is used with the automated BD BACTEC™ MGIT™ 960 and BD BACTEC™ MGIT™ 320 Systems.

Additional Information

The BD BACTEC™ MGIT™ 960 PZA Kit provides susceptibility test results that may be used to determine the antimicrobial agent of choice in treatment of *Mycobacterium tuberculosis*.

SUMMARY AND EXPLANATION

Antimycobacterial susceptibility testing is valuable in the proper treatment of patients with tuberculosis. The treatment of tuberculosis is commonly through a multiple drug regimen that includes the antimycobacterial drug pyrazinamide. It is important that the antimycobacterial drugs prescribed show appropriate activity against *Mycobacterium tuberculosis*, i.e., susceptibility of the isolate to the drug.

Multidrug resistant *Mycobacterium tuberculosis* (MDR-TB) has recently become a serious public health problem.[1] Resistance to any of the primary drugs, including pyrazinamide, makes the disease more difficult and expensive to treat. The rapid detection of these resistant isolates is critical to effective patient management.

Two methods have been widely used for antimycobacterial susceptibility testing. The first method, known as the Method of Proportion,[2] uses Middlebrook and Cohn 7H10 Agar. It compares colony counts on drug-containing and drug-free media. The testing for pyrazinamide requires some modification from the general methods because the drug is active in vitro only at lower pH values.[3] A modification to the method of proportion method was developed using a 7H10 agar medium at pH 5.5, with a drug concentration of 25–50 µg/mL.[4] A limitation of the method is that at a pH of 5.5, many isolates of *M. tuberculosis* either fail to grow or grow poorly. Agar-based methods such as the agar proportion method have not proven to be satisfactory for PZA susceptibility testing because of the failure of many isolates to grow when the agar has been acidified for the PZA test.

The second method, known as the BD BACTEC™ 460TB radiometric susceptibility method,[5] is based on the production of radioactive ^{14}C-labeled carbon dioxide by the growing mycobacteria, manifested by a Growth Index increase in the system. A modification to the BD BACTEC™ 460TB susceptibility method was developed using a modified 7H12 radiometric medium, BD BACTEC™ MGIT™ 960 PZA Medium, with a reduced pH of 6.0.[6] At this pH, PZA activity against mycobacteria can be determined without inhibiting the growth of most *M. tuberculosis* isolates. The BD BACTEC™ 460TB PZA susceptibility test uses a pyrazinamide drug concentration of 100 µg/mL. Susceptibility testing in the BD BACTEC™ 460TB System has proven to be satisfactory and is presently considered the reference method for PZA susceptibility testing. The Clinical and Laboratory Standards Institute (CLSI) recommends the BD BACTEC™ 460TB method for PZA susceptibility testing.[2]

Use of the BD BACTEC™ MGIT™ instrument in combination with the BD BACTEC™ MGIT™ 960 PZA Kit is a non-radiometric method of determining antimycobacterial susceptibility to PZA. The BD BACTEC™ MGIT™ 960 PZA Kit has been developed to allow susceptibility testing at a pyrazinamide concentration of 100 µg/mL. This concentration correlates with the concentration used in the BD BACTEC™ 460TB System.

PRINCIPLES OF THE PROCEDURE

BD BACTEC™ MGIT™ 960 PZA Medium is a tube containing a modified Middlebrook 7H9 Broth, which supports the growth and detection of mycobacteria. The BD BACTEC™ MGIT™ 960 PZA Medium tube contains a fluorescent compound embedded in silicone on the bottom of a 16 x 100 mm round-bottom tube. The fluorescent compound is sensitive to the presence of oxygen dissolved in the broth. The initial concentration of dissolved oxygen quenches the emission from the compound, and little fluorescence can be detected. Later, actively growing and respiring microorganisms consume the oxygen, which allows the compound to fluoresce.

The BD BACTEC™ MGIT™ 960 PZA Kit is a 4–21 day qualitative test. The test is based on growth of the *M. tuberculosis* isolate in a drug-containing tube compared to a drug-free tube (Growth Control). The BD BACTEC™ MGIT™ instrument monitors tubes for increased fluorescence. Analysis of fluorescence in the drug-containing tube compared to the fluorescence of the Growth Control tube is used by the instrument to determine susceptibility results.

The BD BACTEC™ MGIT™ instrument automatically interprets these results and reports a susceptible or resistant result.

BD BACTEC™ MGIT™ 960 PZA Medium is a Partially complete Medium.

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REAGENTS

The BD BACTEC™ MGIT™ 960 PZA Medium tube contains 110 µL of fluorescent indicator and 7 mL of PZA broth. The indicator contains Tris 4,7 - diphenyl-1, 10 phenanthroline ruthenium chloride pentahydrate in a silicone rubber base. The tubes are capped with a polypropylene cap.

Approximate Formula* Per L of Purified Water:

Modified Middlebrook 7H9 broth.....5.9 g
Casein peptone .....1.25 g

BD BACTEC™ MGIT™ 960 PZA Kit contains two lyophilized vials of pyrazinamide and six vials of BD BACTEC™ MGIT™ 960 PZA Supplement.

Approximate Formula* Per Vial Lyophilized drug: Pyrazinamide.....20,000 µg

BD BACTEC™ MGIT™ 960 PZA Supplement contains 15 mL of enrichment

Approximate Formula* Per L Purified Water:

Bovine albumin	50.0 g	Catalase.....	0.03 g
Dextrose	20.0 g	Oleic Acid.....	0.1 g
Polyoxyethylene stearate (POES)	1.1 g		

**Adjusted and/or supplemented as required to meet performance criteria.*

Storage and reconstitution of reagents

BD BACTEC™ MGIT™ 960 PZA Medium - On receipt, store at 2–25 °C. DO NOT FREEZE. Broth should appear clear and colorless. Do not use if turbid. Minimize exposure to light. Tubes stored as labeled prior to use, may be inoculated up to the expiration date.

BD BACTEC™ MGIT™ 960 Pyrazinamide drug vials - On receipt, store the lyophilized drug vials at 2–8 °C. Once reconstituted, use immediately, or the antibiotic solutions may be frozen and stored at -20 °C or colder up to six months, not to exceed the original expiration date. Once thawed, use immediately. Discard unused portions.

BD BACTEC™ MGIT™ 960 PZA Supplement - On receipt, store in the dark at 2–8 °C. Avoid freezing or overheating. Open and use prior to the expiration date. Minimize exposure to light.

Directions For Use

Reconstitute each BD BACTEC™ MGIT™ 960 Pyrazinamide vial with **2.5 mL** of sterile distilled/deionized water to make a stock solution of 8000 µg/mL.

POTENTIALLY INFECTIOUS TEST SPECIMEN: Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. “Standard Precautions”[7-10] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use. For Use by Trained Laboratory Personnel. Do not reuse.

245128 - This Product Contains Dry Natural Rubber.

Working with *M. tuberculosis* growth in culture requires Biosafety Level (BSL) 3 practices, containment equipment and facilities.

Read and follow directions contained in all appropriate package inserts including the BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes.

Prior to use, the user should examine the tubes and vials for evidence of contamination or damage. Discard any tubes or vials if they appear unsuitable. Dropped tubes should be examined carefully. If damage is seen, the tube should be discarded.

In the event of tube breakage: 1) Close the instrument drawers; 2) Turn off the instrument; 3) Vacate the area immediately; 4) Consult your facility/CDC guidelines. An inoculated leaking or broken tube may produce an aerosol of mycobacteria; appropriate handling should be observed.

Autoclave all inoculated BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes prior to disposal.

Dispose of all used reagents and any other contaminated disposable materials following procedures for infectious or potentially infectious waste. It is the responsibility of each laboratory to handle solid and liquid waste according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

INOCULUM PREPARATION

All preparations detailed below must be from pure cultures of *M. tuberculosis*. The laboratory should confirm, by appropriate identification techniques, that the isolate to be tested is a pure culture of *M. tuberculosis*.

Inoculum can be prepared from solid media or from positive BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes. In addition, cultures grown in liquid and on solid media can be used to prepare seed BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes, which can then be used to prepare the inoculum. Each of these options is described below.

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Preparation of the Inoculum from Solid Media

NOTE: It is important to prepare the inoculum according to the following instructions to obtain the appropriate organism concentration for the susceptibility test.

1. Add 4 mL of BD BBL™ Middlebrook 7H9 Broth (or BD BACTEC™ MGIT™ broth) to a 16.5 x 128 mm sterile tube with cap containing 8–10 glass beads.
2. Scrape with a sterile loop as many colonies as possible from growth no more than fourteen days old, trying not to remove any solid medium. Suspend the colonies in the Middlebrook 7H9 Broth.
3. Vortex the suspension for 2–3 minutes to break up the larger clumps. The suspension should exceed a 1.0 McFarland standard in turbidity.
4. Let the suspension sit for 20 minutes without disturbing.
5. Transfer the supernatant fluid to another 16.5 x 128 mm sterile tube with cap (avoid transferring any of the sediment) and let sit for another 15 minutes.
6. Transfer the supernatant fluid (it should be smooth, free of any clumps) to a third 16.5 x 128 mm sterile tube. **NOTE**: The organism suspension should be greater than a 0.5 McFarland standard at this step.
7. Adjust the suspension to a 0.5 McFarland standard by visual comparison to a 0.5 McFarland turbidity standard. Do not adjust below a 0.5 McFarland standard.
8. Dilute 1 mL of the adjusted suspension in 4 mL of sterile saline (1:5 dilution). Use this as the AST inoculum and proceed to "Inoculation Procedure for BD BACTEC™ MGIT™ 960 PZA Kit".

Preparation of the Inoculum from Positive BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes

1. The first day the instrument calls BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes positive is considered Day 0.
2. For the preparation of the test inoculum, positive BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes should be used the day after it first becomes positive on the BD BACTEC™ MGIT™ instrument (Day 1), up to and including the fifth day (Day 5) after instrument positivity. A tube which has been positive longer than five days should be subcultured to fresh BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes containing BD BACTEC™ MGIT™ Growth Supplement and tested on the BD BACTEC™ MGIT™ instrument until positive, and used from one to five days following positivity. See "Preparation of Seed BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes from Liquid Media".
3. If the tube is a Day 1 or Day 2 positive, no dilution is required. Use this as the AST inoculum and proceed to "Inoculation Procedure for BD BACTEC™ MGIT™ 960 PZA Kit Susceptibility Test".
4. If the tube is a Day 3, Day 4, or Day 5 positive, then dilute 1 mL of the positive broth in 4 mL of sterile saline (1:5 dilution). Mix tube thoroughly. Use this as the AST inoculum and proceed to "Inoculation Procedure for BD BACTEC™ MGIT™ 960 PZA Kit Susceptibility Test".

Preparation of Seed BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes from Liquid Media

1. Mix the tube by inversion or vortexing.
2. Make a 1:100 dilution by adding 0.1 mL of the culture into 10 mL of BD BBL™ Middlebrook 7H9 Broth or BD BACTEC™ MGIT™ Broth. Mix well.
3. Add 0.5 mL of this suspension into BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes supplemented with 0.8 mL of BD BACTEC™ MGIT™ Growth Supplement.
4. Cap tightly and gently mix by inverting 2 to 3 times.
5. Enter the tube into a BD BACTEC™ MGIT™ instrument and test until positive.

   NOTE: Time to positivity **must** be ≥4 days for use as AST inoculum. If tube becomes positive in <4 days, return to step 1 and prepare a new seed tube.
6. This tube may now be used from one to five days following positivity. Proceed to "Preparation of the Inoculum from Positive BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes" above.

Preparation of Seed BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes from Solid Media

1. Using a sterile loop, scrape growth from a slant and add to BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes supplemented with 0.8 mL of BD BACTEC™ MGIT™ Growth Supplement.
2. Cap tightly and gently mix by inverting 2 to 3 times.
3. Enter the tube into a BD BACTEC™ MGIT™ instrument and test until positive.

   NOTE: Time to positivity **must** be ≥4 days for use as AST inoculum. If tube becomes positive in <4 days, return to step 1 and prepare a new seed tube.
4. This tube may now be used from one to five days following positivity. Proceed to "Preparation of the Inoculum from a Positive BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes" above.

PROCEDURE

Materials Provided

BD BACTEC™ MGIT™ 960 PZA Kit containing two vials each lyophilized drug and six vials of PZA Supplement (approximately 50 tests per kit).

Materials Required But Not Provided

BD BACTEC™ MGIT™ 960 PZA Medium (25 tubes per carton), ancillary culture media, reagents, quality control organisms and laboratory equipment as required for this procedure.

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Inoculation Procedure for BD BACTEC™ MGIT™ 960 PZA Kit Susceptibility Test:

Important considerations when preparing the PZA AST Set are the proper reconstitution of the lyophilized drug, use of pure culture and the proper dilution of the organism for the Growth Control and PZA tube. It is important to add drug only to the corresponding MGIT™ tube labeled "PZA". Use only the BD BACTEC™ MGIT™ 960 PZA Supplement supplied with the kit and BD BACTEC™ MGIT™ 960 PZA Medium tubes when performing the PZA AST set.

1. Label two 7 mL BD BACTEC™ MGIT™ 960 PZA Medium tubes for each test isolate. Label one as GC (Growth Control), one as PZA. Place the tubes in the correct sequence in the two tube AST set carrier (see BD BACTEC™ MGIT™ Instrument User's Manual).
2. Aseptically add 0.8 mL of BD BACTEC™ MGIT™ 960 PZA Supplement to each tube.
3. Using a micropipette, aseptically pipette 100 µL of the 8,000 µg/mL BD BACTEC™ MGIT™ 960 Pyrazinamide drug solution to the appropriately labeled MGIT™ PZA tube. No PZA drug solution should be added to the appropriately labeled MGIT™ GC tube.

Drug	Concentration of Drug after Reconstitution*	Volume Added to BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes for Test	Final Concentration in BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes
BD BACTEC™ MGIT™ PZA Kit	8,000 µg/mL	100 µL	100 µg/mL*

*PZA must be reconstituted using 2.5 mL sterile/deionized water to achieve the concentration indicated.

4. **Growth Control tube preparation and inoculation:** Aseptically pipette 0.5 mL of the AST inoculum (see "INOCULUM PREPARATION") into 4.5 mL of sterile saline to prepare the **1:10** Growth Control suspension. Mix the Growth Control suspension thoroughly. Inoculate 0.5 mL of the **1:10** Growth Control suspension into the BD BACTEC™ MGIT™ 960 PZA Medium labeled "GC".

   NOTE: It is important to use an appropriately prepared **1:10** dilution for the "GC" tube to ensure accurate AST results and avoid PZA AST set errors.
5. **Drug-containing tube inoculation:** Aseptically pipette 0.5 mL of the AST inoculum (see "INOCULUM PREPARATION") into the BD BACTEC™ MGIT™ 960 PZA Medium labeled "PZA".
6. Tightly recap the tubes. Mix tubes thoroughly by gentle inversion 3 to 4 times.
7. Enter the PZA set into the BD BACTEC™ MGIT™ instrument using the AST set entry feature (refer to the BD BACTEC™ MGIT™ Instrument User's Manual). Ensure that the Growth Control tube is in the first left tube position. Select PZA as the drug in the 2 tube AST set carrier definition when performing the AST set entry.
8. Streak 0.1 mL of the organism suspension to a BD Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) plate. Enclose in a plastic bag. Incubate at 35–37 °C.
9. Check the blood agar plate at 48 hours for bacterial contamination. If the blood agar plate shows no growth, then allow PZA testing to proceed. If the blood agar plate shows growth, discard the PZA set (refer to the BD BACTEC™ MGIT™ Instrument User's Manual) and repeat testing with a pure culture of *Mycobacterium tuberculosis*.

USER QUALITY CONTROL

Upon receipt of a new shipment or lot number of BD BACTEC™ MGIT™ 960 PZA Kit vials or BD BACTEC™ MGIT™ 960 PZA Medium, it is recommended that the control organism shown below be tested. The control organism should be a pure culture and the culture should be prepared according to "INOCULUM PREPARATION" instructions.

The quality control (QC) AST Set should be prepared according to the "Inoculation Procedure for BD BACTEC™ MGIT™ 960 PZA Susceptiblity Test" instructions. Important considerations when preparing the QC AST Set are the proper reconstitution of the lyophilized drug, use of pure culture and the proper dilution of the QC organism for the Growth Control and PZA tubes. It is important to add drug only to the corresponding BD BACTEC™ MGIT™ 960 PZA Medium labeled "PZA".

The same control organism should be run as batch QC once each week when susceptibility testing is performed. Observation of the proper results, as shown below, within 4–20 days indicates that the BD BACTEC™ MGIT™ 960 PZA Kit reagents are ready for use in testing patient isolates.

If the proper results are not observed, do not report patient results. Repeat QC and any patient isolates affected by the initial QC failure. If the repeat QC does not perform as expected, do not report patient results. Do not use the product until you have contacted Technical Services at 1.800.638.8663 or bd.com (United States Only).

Strains	GC	BD BACTEC™ MGIT™ PZA Kit
M. tuberculosis ATCC® 27294	Positive	Susceptible

During the external evaluation of the BD BACTEC™ MGIT™ 960 PZA Kit the average time to result for the control organism was seven days with a range of four to eleven days. The most common causes of QC failures during the external evaluation were over-inoculated PZA Sets and contaminated QC cultures.

RESULTS

The BD BACTEC™ MGIT™ instrument will monitor AST sets until a susceptible or resistant determination is made. Once the set testing is completed, the results are reported by the BD BACTEC™ MGIT™ instrument (refer to the BD BACTEC™ MGIT™ Instrument User's Manual). The BD BACTEC™ MGIT™ instrument will report an AST Set result as an Error ("X"), no susceptibility interpretation, when certain conditions occur that may affect the test results. Conditions that may result in an Error ("X") result are described in Section 7–Troubleshooting of the BD BACTEC™ MGIT™ Instrument User's Manual.

It is important to include the test method, drug name and concentration when reporting results. The Pulmonary and/or Infectious Disease specialist in TB control should be consulted concerning the appropriate therapeutic regimen and dosages.

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Mono-resistance to pyrazinamide is uncommon, therefore in the event of unexpected resistant results, verify purity and identification of the isolate tested as *M. tuberculosis*. Guidelines for mycobacterial purity checks can be found in the CLSI M24 standard.[2]

BD BACTEC™ MGIT™ 960 PZA Kit result reporting

Drug (concentration)	BD BACTEC™ MGIT™ System result	Recommended Report	Action
PZA (100 µg/mL)	Susceptible	Isolate tested with BD BACTEC™ MGIT™ 960 PZA Kit [PZA/100 µg/mL] and result is susceptible.	No action.
	Resistant	Isolate tested with BD BACTEC™ MGIT™ 960 PZA Kit [PZA/100 µg/mL] and result is resistant.	If isolate is mono-resistant to PZA, confirm that isolate tested is a pure culture of *Mycobacterium tuberculosis*.
	Error "X"	No report.	Repeat test.

LIMITATIONS OF THE PROCEDURE

The BD BACTEC™ MGIT™ 960 PZA Kit does not interpret the degree of susceptibility of the isolate being tested. Results are reported as either susceptible or resistant.

The BD BACTEC™ MGIT™ 960 PZA Kit can only be performed using a BD BACTEC™ MGIT™ instrument. The PZA Sets cannot be read manually.

Use only pure cultures of *M. tuberculosis*. Cultures that are contaminated or that may contain multiple species of mycobacteria may give erroneous results and should not be tested. Direct testing from clinical specimens is not recommended.

Suspensions made from solid media must be allowed to settle for the prescribed times prior to standardization. Inoculum preparations made from solid media should be visually compared to a 0.5 McFarland turbidity standard; failure to do so may give inaccurate results or cause an AST Set error.

Failure to use the 1:5 dilution of the organism suspension, when indicated, to inoculate the drug containing tubes may give inaccurate results.

Failure to use a 1:10 dilution of the organism suspension for the inoculation of the Growth Control tube may give inaccurate results or cause an AST Set error.

Failure to reconstitute the PZA drug with the appropriate volume of sterile distilled / deionized water may give inaccurate results.

Thorough mixing of inoculated tubes is important. Failure to mix the tubes adequately may lead to false resistant results.

Failure to load the tubes of the AST Set into the AST Set Carrier in the proper sequence may give inaccurate results. Failure to select the appropriate set carrier drug definition may result in invalid or inaccurate results.

Failure to load the AST Set into the instrument correctly will result in an anonymous condition that must be resolved within 8 hours. If condition is not resolved within 8 hours, the AST Set must be discarded and set up again.

Failure to use the BD BACTEC™ MGIT™ 960 PZA Supplement in the PZA AST set may give inaccurate results. DO NOT add BD BACTEC™ MGIT™ 960 SIRE Supplement or BD BACTEC™ MGIT™ Growth Supplement to the PZA AST set.

Failure to use BD BACTEC™ MGIT™ 960 PZA Medium for the PZA AST set may give inaccurate results. DO NOT substitute BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes for BD BACTEC™ MGIT™ 960 PZA Medium.

EXPECTED VALUES

A total of 118 clinical isolates of *M. tuberculosis* were tested with the BD BACTEC™ MGIT™ 960 PZA Kit at four geographically diverse sites. The testing included both fresh clinical and subcultured isolates from both liquid and solid culture sources. A total of 228 PZA susceptibility tests (liquid and solid) were performed.

During the external evaluation of the BD BACTEC™ MGIT™ 960 PZA Kit, there were nine PZA tests from clinical isolates that required repeat testing due to contamination (six isolates) or overinoculation/procedural errors (three isolates).

The average time-to-result for the BD BACTEC™ MGIT™ 960 PZA Kit is seven days with a range from four to seventeen days. The data are shown in Figure 1 at end of insert.

PERFORMANCE CHARACTERISTICS

Analytical Studies

Liquid and Solid Media AST Inoculum Ranges

Liquid media - The recommended procedure for preparing a PZA Set from positive BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes uses a direct inoculum on Day 1 and Day 2 post-positivity and a dilute (1:5) inoculum on Day 3 to Day 5 post-positivity. Internal studies show that inocula prepared from a Day 1 to Day 5 positive MGIT™ 7 mL tube range between 2.0×10^4 to 7.5×10^6 CFU/mL.

Solid media - The recommended procedure for preparing a PZA Set from growth on solid media (up to 14 days after first visible growth is seen) uses a 1:5 dilution of an organism suspension equivalent to a 0.5 McFarland Standard. Internal studies show that inocula prepared from solid medium culture range between 2.1×10^5 to 3.9×10^6 CFU/mL.

Lot Reproducibility

Lot reproducibility was evaluated using twenty-five *M. tuberculosis* strains (including three ATCC strains). Each strain was tested in triplicate with the BD BACTEC™ MGIT™ 960 PZA Kit. Each replicate represented a separate test condition differentiated by lot of PZA drug, PZA supplement and PZA medium used (three lots each).

Observed results were compared to the expected results. The overall reproducibility for the BD BACTEC™ MGIT™ 960 PZA Kit is 96.8%.

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CDC Challenge Panel Testing

The performance of the BD BACTEC™ MGIT™ 960 PZA Kit was evaluated using a panel of challenge strains obtained from the Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA. The panel consisted of nine strains of *M. tuberculosis* with known susceptibility patterns (using BD BACTEC™ 460TB). The panel was tested in triplicate with the BD BACTEC™ MGIT™ 960 PZA Kit. The BD BACTEC™ MGIT™ 960 PZA Kit results were compared to the CDC expected results. The overall agreement with CDC expected results for the BD BACTEC™ MGIT™ 960 PZA Kit is 98.7%.

Clinical Evaluation

The BD BACTEC™ MGIT™ 960 PZA Kit was evaluated at four geographically diverse clinical sites composed of regional reference centers and university hospital-based laboratories, including two ex-US sites. The BD BACTEC™ MGIT™ 960 PZA Kit was compared to the BD BACTEC™ 460TB PZA susceptibility test method.

Reproducibility Testing

The reproducibility of the BD BACTEC™ MGIT™ 960 PZA Kit was evaluated at the clinical sites using a panel of five qualified strains. The BD BACTEC™ MGIT™ 960 PZA Kit test results were compared to the expected results. The overall reproducibility for the BD BACTEC™ MGIT™ 960 PZA Kit is 94%.

CDC Challenge Panel Testing

The performance of the BD BACTEC™ MGIT™ 960 PZA Kit was evaluated at each of the four clinical sites using a panel of challenge strains obtained from the Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA. The panel consisted of nine strains of *M. tuberculosis* with known susceptibility patterns (using BD BACTEC™ 460TB). Of the thirty-six PZA results collected with the BD BACTEC™ MGIT™ 960 PZA Kit, thirty-three agreed with the CDC expected results. The calculated percent agreement to the CDC expected results for the BD BACTEC™ MGIT™ 960 PZA Kit is 91.7%.

Clinical Isolate Testing

A total of 118 clinical isolates of *M. tuberculosis* were tested with the BD BACTEC™ MGIT™ 960 PZA Kit and the BD BACTEC™ 460TB PZA susceptibility test. This included testing of both fresh clinical and subcultured isolates from both liquid and solid culture sources. This generated a total of 228 test results.

Table 1 presents the results from clinical isolate testing for PZA drug at 100 µg/mL from liquid source cultures, from solid source cultures and both source cultures combined.

Table 1: Clinical Isolate Results – BD BACTEC™ MGIT™ 960 PZA Kit compared to BD BACTEC™ 460TB susceptibility test

		BD BACTEC™ 460TB System		BD BACTEC™ MGIT™ 960 System			
		Expected PZA Results		Susceptible Results		Resistant Results	
Source	# Tests	S	R	# agree	Category agreement % (95% CI)	# agree	Category agreement % (95% CI)
LIQUID	112	89	23	88	98.9% (93.9–100)	22	95.7% (78.1–99.9)
SOLID	113*	90	23	88	97.8% (92.2–99.7)	20	87.0% (66.4–97.2)
ALL	225*	179	46	176	98.3% (95.2–99.7)	42	91.3% (79.2–97.6)

*Three BD BACTEC™ 460TB borderline results are not included in this table.

All isolates with discordant BD BACTEC™ MGIT™ 960 PZA Kit test results were tested using the BD BACTEC™ 460TB PZA susceptibility test at two independent sites. Discordant results were those strains where the BD BACTEC™ MGIT™ 960 PZA Kit test result differed from the BD BACTEC™ 460TB PZA test result. Borderline results are not included in the performance calculations for the BD BACTEC™ MGIT™ 960 PZA Kit.

Of the four discordant PZA susceptible (S-BACTEC™ MGIT™ 960, R-BACTEC™ 460TB) isolates tested, one had susceptible results from both independent sites and the other three had resistant results from both independent sites. Of the three discordant PZA resistant (R-BACTEC™ MGIT™ 960, S-BACTEC™ 460TB) isolates tested, all isolates had susceptible results from both independent sites.

Two of the three BD BACTEC™ 460TB borderline PZA results (S-BACTEC™ MGIT™ 960, B-BACTEC™ 460TB) had susceptible results from both independent sites. One of the three BD BACTEC™ 460TB borderline PZA results (R-BACTEC™ MGIT™ 960, B-BACTEC™ 460TB) had one independent site determine a susceptible result. The other independent site determined a borderline result.

AVAILABILITY

Catalog Number	Description
245128	BD BACTEC™ MGIT™ 960 PZA Kit.
245115	BD BACTEC™ MGIT™ 960 PZA Medium, 25 tests.

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Technical Service and Support: In the United States contact BD at 1.800.638.8663 or bd.com.

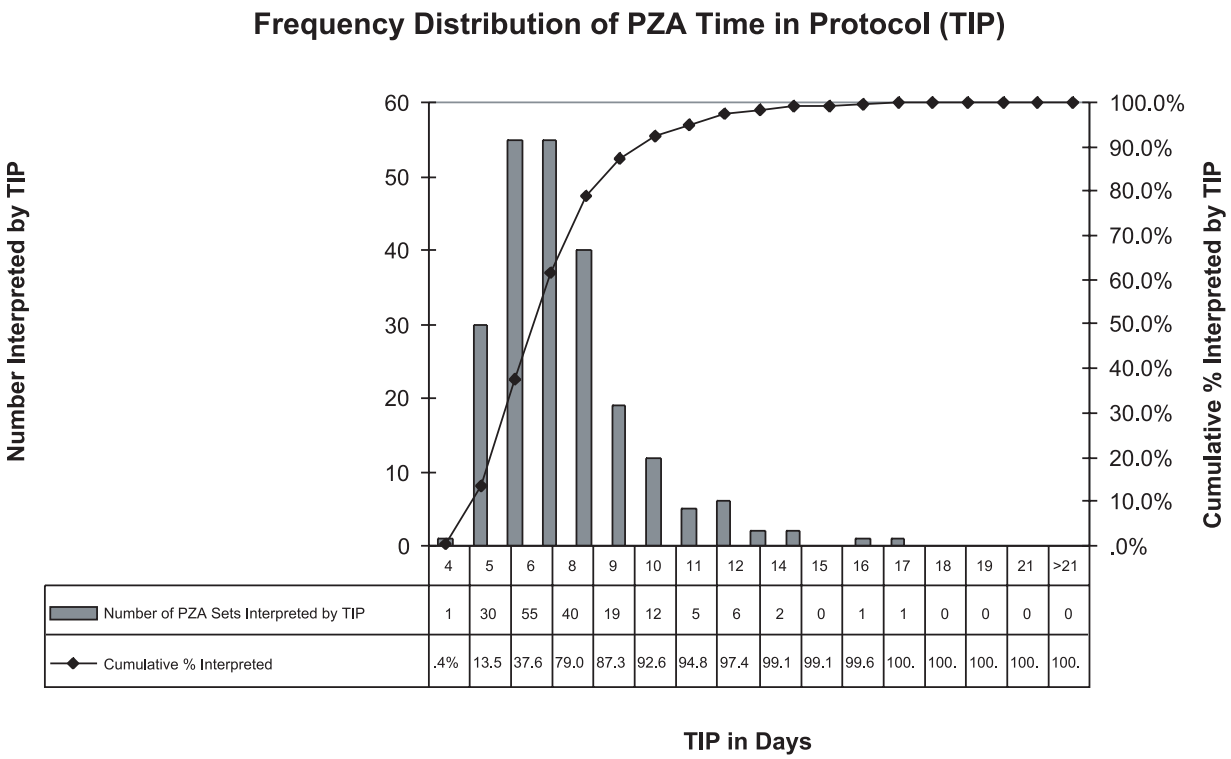
For regions outside of the United States, contact your local BD representative or bd.com.

EU Only: Users shall report any serious incident related to the device to the Manufacturer and National Competent Authority.

Outside EU: Contact your local BD representative for any incident or inquiry related to this device.

Refer to the Eudamed website: https://ec.europa.eu/tools/eudamed for Summary of Safety and Performance.

Figure 1: Distribution of BD BACTEC™ MGIT™ 960 PZA AST Time to Result



TIP in Days	4	5	6	8	9	10	11	12	14	15	16	17	18	19	21	>21
Number of PZA Sets Interpreted by TIP	1	30	55	40	19	12	5	6	2	0	1	1	0	0	0	0
Cumulative % Interpreted	.4%	13.5	37.6	79.0	87.3	92.6	94.8	97.4	99.1	99.1	99.6	100.	100.	100.	100.	100.

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Change History

Revision	Date	Change Summary
04	2019-09	Converted printed instructions for use to electronic format and added access information to obtain the document from bd.com/e-labeling.
05	2019-10	Added Chart of Frequency Distribution of PZA Time in Protocol (TIP), inadvertently not included in the previously undistributed version.
06	2023-05	Added CE notified body number (2797) for IVDR 2017/746. Added Do not reuse and Keep away from light symbols. Added Safe Disposal section. Updated Warning and Precautions. Added “245128 - This Product Contains Dry Natural Rubber” statement. Added Eudamed Link statement. Updated Symbols Glossary. Removed eIFU/url, Key code, and Phone numbers from back page. Updated EC REP Address. Updated Australian Sponsor Address, and added New Zealand Sponsor Address. Added CH REP symbol with address. Updated storage instruction section - Once reconstituted, use immediately, or the antibiotic solutions may be frozen and stored at -20 °C or colder up to six months, not to exceed the original expiration date. Added EU and Swiss Importer addresses with symbol. Added US Patent statement. Updated Product Names and Corrected Grammatical updates. Updated Summary and Explanation, Inoculum Preparation sections. Updated all ‘BD BACTEC™ MGIT™ Tubes’ to “BD BACTEC™ MGIT™ Mycobacteria Growth Indicator Tubes”.

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SYMBOLS GLOSSARY

Please refer to product labeling for applicable symbols.

Symbol	Meaning
	Manufacturer
EC REP	Authorized representative in the European Community
CH REP	Authorised representative in Switzerland
	Date of manufacture
	Use-by date
LOT	Batch code
REF	Catalogue number
SN	Serial number
STERILE	Sterile
STERILE A	Sterilized using aseptic processing techniques
STERILE EO	Sterilized using ethylene oxide
STERILE R	Sterilized using irradiation
STERILE	Sterilized using steam or dry heat
	Do not resterilize
NON STERILE	Non-sterile
	Do not use if package is damaged and consult *instructions for use*
STERILE	Sterile fluid path
STERILE EO	Sterile fluid path (ethylene oxide)
STERILE R	Sterile fluid path (irradiation)
	Fragile, handle with care
	Keep away from sunlight
	Keep dry
	Lower limit of temperature
	Upper limit of temperature
	Temperature limit
	Humidity limitation
	Biological risks
	Do not re-use
	Consult *instructions for use* or consult electronic *instructions for use*
	Caution
LATEX	Contains or presence of natural rubber latex
IVD	In vitro diagnostic medical device
CONTROL -	Negative control
CONTROL +	Positive control
Σ	Contains sufficient for <*n*> tests
	For IVD performance evaluation only
	Non-pyrogenic
#	Patient number
	This way
	Do not stack

Symbol	Meaning
	Single sterile barrier system
PHT DEHP BBP	Contains or presence of phthalate: combination of bis(2-ethylhexyl) phthalate (DEHP) and benzyl butyl phthalate (BBP)
	Collect separately Indicates separate collection for waste of electrical and electronic equipment required.
CE	CE marking; Signifies European technical conformity
	Device for near-patient testing
	Device for self-testing
Rx Only	This only applies to US: "Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner."
CC	Country of manufacture "CC" shall be replaced by either the two letter or the three letter country code.
	Collection time
	Cut
	Peel here
	Collection date
	Keep away from light
H_2	Hydrogen gas is generated
	Perforation
	Start panel sequence number
	End panel sequence number
	Internal sequence number
/	<Box #> / <Total Boxes>
MD	Medical device
	Contains hazardous substances
	Ukrainian conformity mark
FC	Meets FCC requirements per 21 CFR Part 15
c UL us	UL product certification for US and Canada
UDI	Unique device identifier
	Importer
	Place patient label in framed area only
MR	Magnetic resonance (MR) safe
MR	Magnetic resonance (MR) conditional
MR	Magnetic resonance (MR) unsafe
For use with	For use with
This Product Contains Dry Natural Rubber	This Product Contains Dry Natural Rubber
For Export Only	For Export Only
Instruments	Instruments

Note: Text layout in symbols is determined by label design.

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