PROVIDENCE
Sacred Heart
Medical Center &
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M. tuberculosis Susceptibility Testing BACTEC™ MGIT™ 960 Procedure

Department of Microbiology

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Table of Contents

1.0 Purpose or Principle

Two methods have been widely used for antimycobacterial susceptibility testing. The first method, the Method of Proportion (MOP), uses Middlebrook and Cohn 7H10 Agar. It compares colony counts on drug-containing and drug-free media. Resistance to a drug is detected when 1% or more of the bacterial population is resistant to the drug concentration tested. Results are generally available after 21 days of incubation. The second method, the BACTEC 460TB radioactive susceptibility method, generally takes from 4 to 12 days. It 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.

Historically, the MOP procedure has included susceptibility testing of *M. tuberculosis* using two concentrations of antimicrobials. CLSI continues to recommend that the MOP test procedure include two concentrations of the primary drugs for testing, except rifampin. The recommended low concentrations for the MOP procedure are the critical concentrations for these drugs. The critical concentration is defined as the drug concentration that allows the interpretation of a result as either resistant or susceptible. An isolate is determined resistant if 1% or more of the test population grows in the presence of the critical concentration of the drug. The high drug concentration is used to profile the degree of resistance within the population. The result provides information to the physician to assist in determining whether a modification to the therapy regimen is necessary.

The BACTEC MGIT 960 SIRE Kit is a rapid qualitative procedure for susceptibility testing of *Mycobacterium tuberculosis*, from culture, to streptomycin (STR), isoniazid (INH), rifampin (RIF), and ethambutol (EMB). The BACTEC MGIT 960 SIRE test was developed with critical concentrations slightly lower than the critical concentrations used in the MOP procedure in order to avoid false susceptibility. This is most apparent for streptomycin where many isolates are close to the recommended critical concentration as performed by the MOP. For this reason, a second, higher drug concentration was developed for streptomycin and isoniazid. A susceptible result at the critical concentration can be reported and no other testing is necessary. Isolates that are resistant at the critical concentration of streptomycin or isoniazid should be tested at a higher drug concentration. The BACTEC MGIT 960 STR 4.0 Kit and the BACTEC MGIT 960 INH 0.4 Kit are for testing at higher drug concentrations. Testing of resistant isolates at a higher concentration is important to identify those that exhibit low-level resistance, i.e., resistant at the critical concentration and susceptible at the high concentration. The high concentrations in the BACTEC MGIT 960 were designed to be lower than the concentrations used in MOP. This design is such that a resistant result, especially for streptomycin, may not always correlate to a resistant result at the high concentration in MOP. In the event that a streptomycin result is obtained that is resistant at the high concentration, an alternate method of testing at this concentration should be performed. The end result of the alternate method is intended to provide information to the physician to assist in determining whether a modification to the therapy regimen is necessary.

The BBL MGIT 7 mL Mycobacterial Growth Indicator Tube contains a modified Middlebrook 7H9 Broth that supports the growth and detection of mycobacteria. The MGIT tube contains a fluorescent compound embedded in silicone on the bottom of the 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 and allow the compound to fluoresce.

The BACTEC MGIT 960 SIRE Kit is a 4-13 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 BACTEC 960 instrument continually monitors tubes for increased fluorescence. Analysis of fluorescence in the drug-containing tube as compared to the fluorescence of the Growth Control tube is used by the instrument to determine susceptibility results. The BACTEC MGIT 960 instrument automatically interprets these results, and reports a susceptible or resistant result.

The BACTEC MGIT 960 PZA Medium is a tube containing a modified Middlebrook 7H9 Broth, which supports the growth and detection of mycobacteria at a reduced pH of 5.9. The BACTEC MGIT 960 PZA Medium tube contains a fluorescent compound embedded in silicone on the bottom of the tube and functions the same as a BACTEC MGIT tube in terms of growth and detection. The BACTEC MGIT 960 PZA Kit is a 4-21 day qualitative test that functions the same as described above for the 960 SIRE Kit.

2.0 Clinical Significance

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 streptomycin, isoniazid, rifampin, ethambutol and pyrazinamide. It is important that the drugs prescribed show appropriate activity against *Mycobacterium tuberculosis*.

3.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel familiarized and trained on all levels of the operation of the BACTEC™ MGIT™ testing platform. Testing includes but is not limited to: instrument performance checks, basic troubleshooting, QC checks, administrative tasks and record keeping of information vital to verification of instrument and technical proficiency in accordance with the department SOP. Records are to be kept within the employee's record in the department of continued competence and proficiency on the equipment. Performance reviews of technical personnel are to be carried out annually.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All isolates must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines. Working with *M. tuberculosis* in culture requires Biosafety Level (BSL) 3 practices, containment equipment and facilities. Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Bloodborne pathogens
- Airborne pathogens

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. In the event of tube breakage on the BACTEC 960:

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

To perform this procedure, you must use:

- Gloves
- Laboratory Coat
- N95 Respirator
- Biological safety cabinet

Disinfectant following procedure:

- Diluted bleach (10% solution made fresh daily or mixed just before use)

5.0 Specimen Requirements

All preparations 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*.

5.1.1 Preparation of Inoculum from Solid Media

1. Add 4 mL of BBL Middlebrook 7H9 Broth (or BBL MGIT broth) to a 16.5 x 128 mm sterile tube with a cap containing 8 – 10 glass beads.
2. Scrape with a sterile loop as many colonies as possible from growth no more than 14 d old, trying not to remove any solid medium. Suspend the colonies in the Middlebrook 7H9 Broth.
3. Vortex the suspension for 2 – 3 min to break up the larger clumps. The suspension should exceed a 1.0 McFarland standard turbidity.
4. Let the suspension sit for 20 min without disturbing.
5. Transfer the supernatant fluid to another 16.5 x 128 mm sterile tube with a cap (avoid transferring any of the sediment) and let sit for another 15 min.
6. Transfer the supernatant fluid (it should be smooth, free of 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 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). Proceed to the Inoculation Procedure for Susceptibility Test.

5.1.2 Preparation of the Inoculum from a Positive BACTEC MGIT 7 mL Tube

1. The first day of an instrument positive MGIT tube is considered Day 0.
2. For the preparation of the test inoculum, a positive 7 mL MGIT tube should be used the day after it first becomes positive on the BACTEC MGIT 960 instrument (Day 1), up to and including the fifth day (Day 5) after instrument positivity. A tube which has been positive longer than 5 days should be subcultured to a fresh 7 mL MGIT tube containing BACTEC MGIT 960 Growth Supplement and tested on the BACTEC MGIT 960 instrument until positive, and used from one to five days following positivity. See Preparation of a Seed MGIT Tube from Liquid Media below.
3. If the tube is Day 1 or Day 2 positive, use the MGIT broth suspension for the inoculation procedures. Mix well. Proceed to Inoculation Procedure for Susceptibility Test.
4. If the tube is a Day 3, Day 4, or Day 5 positive, mix well then dilute 1 mL of the positive broth in 4 mL of sterile saline (1:5 dilution). Mix the tube thoroughly. Use the diluted suspension for the inoculation procedures. Proceed to Inoculation Procedure for Susceptibility Test.

5.1.3 Preparation of a Seed MGIT Tube from Liquid Media

1. Mix the positive MGIT tube by inversion or vortexing.
2. Make a 1:100 dilution by adding 0.1 mL of the culture into 10 mL of BBL Middlebrook 7H9 Broth or BBL MGIT Broth. Mix well.
3. Add 0.5 mL of this suspension to a 7 mL MGIT tube supplemented with 0.8 mL of BACTEC MGIT 960 Growth Supplement.
4. Cap tightly and gently mix by inverting 2 - 3 times.
5. Enter the tube into a BACTEC MGIT 960 instrument and test until positive.

   NOTE: Time to positivity must be $\geq$ 4 days for use as AST inoculum. If tube becomes positive in < 4 days, return to first step 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 a Positive BACTEC MGIT 7 mL Tube above.

5.1.4 Preparation of a Seed MGIT Tube from Solid Media

1. Using a sterile loop, scrape growth from a slant and add to 7 mL MGIT tube supplemented with 0.8 mL of BACTEC MGIT 960 Growth Supplement.
2. Cap tightly and gently mix by inverting 2 – 3 times.
3. Enter the tube into a BACTEC MGIT 960 instrument and test until positive.

   NOTE: Time to positivity must be $\geq$ 4 days for use as AST inoculum. If tube becomes positive in < 4 days, return to first step 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 BACTEC MGIT 7 mL Tube above.

6.0 Procedure Information and Instructions

6.1 Materials

6.1.1 Equipment and/or Testing System

- BACTEC™ MGIT™ 960, Becton Dickinson
- Micropipette (100 – 1,000 µL range) – calibrated annually

6.1.2 Consumables

- Micropipette tips specific to instrument in use.
- Sterile vials to aliquot and freeze reconstituted antimicrobials
- Sterile 50-mL conical tubes

6.1.3 Reagents & Media

- **BACTEC MGIT 960 SIRE Kit** contains one each lyophilized vials of streptomycin, isoniazid, rifampin, and ethambutol and eight vials of SIRE Supplement. Upon receipt, store at 2 - 8°C. Approximate formula per vial lyophilized drug:
  - Streptomycin 332 µg
  - Isoniazid 33.2 µg
  - Rifampin 332 µg
  - Ethambutol 1660 µg

  Reconstitute each BACTEC MGIT 960 **SIRE Kit** Streptomycin, Isoniazid, Rifampin, and Ethambutol lyophilized drug vial with **4 mL** of sterile/deionized water. Once reconstituted, the antibiotic solutions may be frozen in aliquots and stored at - 20°C or colder up to six months, not to exceed the original expiration date. Once thawed, use the antibiotic solution immediately. Discard unused portions.

  Final stock solutions:
  - Streptomycin 83 µg/mL
  - Isoniazid 8.3 µg/mL
  - Rifampin 83 µg/mL
  - Ethambutol 415 µg/mL
- **BACTEC MGIT 960 STR 4.0 Kit** contains one vial of lyophilized streptomycin and two vials of SIRE Supplement. Upon receipt, store at 2 - 8°C.

  Approximate formula per vial lyophilized drug:
  - Streptomycin 664 µg

  Reconstitute each BACTEC MGIT 960 **STR 4.0** Kit Streptomycin lyophilized drug vial with **2 mL** of sterile/deionized water. Once reconstituted, the antibiotic solution may be frozen and stored at - 20°C or colder up to six months, not to exceed the original expiration date. Once thawed, use the antibiotic solution immediately. Discard unused portions.

  Final stock solution:
  - Streptomycin 332 µg/mL

- **BACTEC MGIT 960 INH 0.4 Kit** contains one vial of lyophilized isoniazid and two vials of SIRE Supplement. Upon receipt, store at 2 - 8°C.

  Approximate formula per vial lyophilized drug:
  - Isoniazid 66.4 μg

  Reconstitute each BACTEC MGIT 960 **INH 0.4** Kit Streptomycin lyophilized drug vial with **2 mL** of sterile/deionized water. Once reconstituted, 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 the antibiotic solution immediately. Discard unused portions.

  Final stock solution:
  - Isoniazid 33.2 μg/mL

- **BACTEC MGIT 960 SIRE Supplement** contains 20 mL Middlebrook OADC enrichment. Upon receipt, store in dark at 2 - 8°C. Avoid freezing or overheating. Open and use prior to the expiration date. Minimize exposure to light.

  Approximate formula per L purified water:
  - Bovine albumin 50.0 g
  - Dextrose 20.0 g
  - Catalase 0.03 g
  - Oleic Acid 0.6 g

- **BACTEC MGIT 960 PZA Kit** contains two lyophilized vials of pyrazinamide and six vials of PZA Supplement. Upon receipt, store in dark at 2 - 8°C. Avoid freezing or overheating. Open and use prior to the expiration date. Minimize exposure to light.

  Approximate formula per vial lyophilized drug:
  - Pyrazinamide 20,000 μg

  Reconstitute each BACTEC MGIT 960 **PZA** lyophilized drug vial with **2.5 mL** of sterile distilled/deionized water. Once reconstituted, the antibiotic solutions may be frozen in aliquots and stored at - 20°C or colder up to six months, not to exceed the original expiration date. Once thawed, use the antibiotic solution immediately. Discard unused portions.

  Final stock solution:
  - Pyrazinamide 8,000 μg/mL

- **BACTEC MGIT 960 PZA Supplement** contains 15 mL of enrichment. Upon receipt, store in dark at 2 - 8°C. Avoid freezing or overheating. Open and use prior to the expiration date. Minimize exposure to light.

  Approximate formula per L purified water:
  - Bovine albumin 50.0 g
  - Dextrose 20.0 g
  - Polyoxyethylene stearate 1.1 g
  - Catalase 0.03 g
  - Oleic Acid 0.1 g

- **BACTEC MGIT 960 Mycobacteria Growth Indicator Tubes.** Upon receipt, store at 2 - 25°C. Do not freeze. Broth should appear clear and colorless. Do not use if turbid. Minimize exposure to light. Properly stored tubes may be used until expiration date.

  Approximate formula per L purified water:
  - Modified Middlebrook 7H9 broth 5.9 g
  - Casein peptone 1.25 g

- **BACTEC MGIT 960 PZA Medium** (pH adjusted to 5.9). Upon receipt, store at 2 - 25°C. Do not freeze. Broth should appear clear and colorless. Do not use if turbid. Minimize exposure to light. Properly stored tubes may be used until expiration date.

  Approximate formula per L purified water:
  - Modified Middlebrook 7H9 broth 5.9 g
  - Casein peptone 1.25 g

6.1.4 Control Organism

- *Mycobacterium tuberculosis* ATCC 27294

6.2 Interfering Substances

Cultures that are contaminated or that may contain multiple species of mycobacteria may give erroneous results and should not be tested.

7.0 Step by Step Instructions

7.1 SIRE Kit Susceptibility Test

1. Label five 7-mL MGIT tubes for each test isolate. Label one as GC (Growth Control), one as STR, one as INH, one as RIF, and one as EMB. Place the tubes in the correct sequence in the appropriate size AST SET Carrier.
2. Aseptically add 0.8 mL of BACTEC MGIT SIRE Supplement to each tube. NOTE: It is important to use the supplement supplied with the kit.
3. Aseptically pipette, using a micropipette, 100 µL of 83 µg/mL MGIT STR solution to the appropriately labeled MGIT tube. Aseptically pipette 100 µL of 8.3 µg/mL MGIT INH solution to the appropriately labeled MGIT tube. Aseptically pipette 100 µL of 83 µg/mL MGIT RIF solution to the appropriately labeled MGIT tube. Aseptically pipette 100 µL of 415 µg/mL MGIT EMB solution to the appropriately labeled MGIT tube. It is important to add the correct drug to the corresponding tube. No antibiotics should be added to the MGIT GC tube.

Drug	Concentration of Drug after Reconstitution*	Volume Added to MGIT Tubes for Test	Final Concentration in MGIT Tubes
MGIT STR	83 µg/mL	100 µL	1.0 µg/mL
MGIT INH	8.3 µg/mL	100 µL	0.1 µg/mL
MGIT RIF	83 µg/mL	101 µL	1.0 mg/mL
MGIT EMB	415 µg/mL	102 µL	5.0 µg/mL

*These drugs must be reconstituted using 4 mL sterile/deionized water to achieve concentrations indicated.

4. **Growth Control tube preparation and inoculation**: Aseptically pipette 0.1 mL of the organism suspension (see "Inoculum Preparation") into 10 mL of sterile saline to prepare the 1:100 Growth Control suspension. Mix the Growth Control suspension thoroughly. Inoculate 0.5 mL of the 1:100 Growth Control suspensions into the MGIT tube labeled "GC."
5. **Drug-containing tube inoculation**: Aseptically pipette 0.5 mL of the organism suspension (see "Inoculum Preparation") into each of the four remaining tubes (STR, INH, RIF, EMB).
6. Tightly recap the tubes. Mix tubes thoroughly by gentle inversion three to four times.
7. Enter the AST set into the BACTEC MGIT 960 using the AST set entry feature (refer to the BACTEC MGIT 960 User's Manual, AST Instructions). Ensure that the order of the tubes in the AST Set carrier conforms to the set carrier definitions selected when performing the AST set entry feature.
8. Streak 0.1 mL of the organism suspension to a BAP. Enclose in a plastic bag. Incubate at 35 – 37°C.

Check the BAP at 48 h for bacterial contamination. If the BAP shows no growth, then allow AST testing to proceed. If the BAP shows growth, discard AST set (refer to the BACTEC MGIT 960 User's Manual, AST Instruction) and repeat testing with pure culture.

7.2 STR 4.0 and INH 0.4 Kit Susceptibility Test

It is recommended if resistance occurs at the critical concentration, a susceptibility profile test be performed which, at a minimum, tests the high concentration of the drug to which the isolate was originally resistant.

Isolate Source: The isolate used for this testing must have been prepared as described in "Inoculum Preparation." A seed tube may be prepared from the drug-free Growth Control tube from the previously tested AST set of the isolate, by inoculating 0.5 mL to a fresh MGIT 7 mL tube containing BACTEC MGIT 960 Growth supplement. Once the seed tube is instrument positive, proceed as described in Inoculum Preparation: "Preparation of the Inoculum from a Positive MGIT tube."

1. Label enough 7-mL MGIT tubes for the test isolate to have a MGIT GC (Growth Control) and a MGIT drug tube for each antibiotic tested. Place the tubes in the correct sequence in the appropriate size AST Set Carrier.
2. Aseptically add 0.8 mL of BACTEC MGIT SIRE Supplement to each tube. NOTE: It is important to use the supplement supplied with the kit.
3. Aseptically pipette, using a micropipette, 100 μL of the drug solution to the appropriately labeled MGIT tube. It is important to add the correct drug to the corresponding tube. No antibiotics should be added to the MGIT GC tube.

Drug	Concentration of Drug after Reconstitution*	Volume Added to MGIT Tubes for Test	Final Concentration in MGIT Tubes
MGIT STR 4.0	332 μg/mL	100 μL	4.0 μg/mL
MGIT INH 0.4	33.2 μg/mL	100 μL	0.4 μg/mL

*These drugs must be reconstituted using 4 mL sterile/deionized water to achieve concentrations indicated.

4. **Growth Control tube preparation and inoculation**: Aseptically pipette 0.1 mL of the organism suspension (see "Inoculum Preparation") into 10 mL of sterile saline to prepare the 1:100 Growth Control suspension. Mix the Growth Control suspension thoroughly. Inoculate 0.5 mL of the 1:100 Growth Control suspension into the MGIT tube labeled "GC."
5. **Drug-containing tube inoculation**: Aseptically pipette 0.5 mL of the organism suspension (see "Inoculum Preparation") into each of the drug tubes.
6. Tightly recap the tubes. Mix tubes thoroughly by gentle inversion three to four times.
7. Enter the AST set into the BACTEC MGIT 960 using the AST set entry feature (refer to the BACTEC MGIT 960 User's Manual, AST Instructions). Ensure that the order of the tubes in the AST Set carrier conforms to the set carrier definitions selected when performing the AST set entry feature.
8. Streak 0.1 mL of the organism suspension to a BAP. Enclose in a plastic bag. Incubate at 35 - 37°C.
9. Check the BAP at 48 h for bacterial contamination. If the BAP shows no growth, then allow AST testing to proceed. If the BAP shows growth, discard AST set (refer to the BACTEC MGIT 960 User's Manual, AST Instruction) and repeat testing with pure culture.

NOTE: The susceptibility test may be configured in a variety of formats. For example, a five tube carrier set containing only the critical concentrations can be configured into the system. A variety of other tube carrier sets can be configured depending on the optional profile tests being run (refer to the BACTEC MGIT 960 User's Manual, AST Instructions)

7.3 PZA Kit Susceptibility Test

1. Label two 7-mL BACTEC MGIT 960 PZA Medium tubes for each test isolate. Label one as GC (Growth Control), and one as PZA. Place the tubes in the correct sequence in the two-tube AST set carrier.
2. Aseptically add 0.8 mL of BACTEC MGIT 960 PZA Supplement to each tube.
3. Using a micropipette, aseptically transfer 100 μL of 8,000 μg/mL BACTEC MGIT 960 PZA 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 MGIT Tubes for Test	Final Concentration in MGIT Tubes
MGIT PZA	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 MGIT tube labeled "GC."
5. Drug-containing tube inoculation: Aseptically pipette 0.5 mL of the organism suspension (see "Inoculum Preparation") into the MGIT tube labeled "PZA."
6. Tightly recap the tubes. Mix tubes thoroughly by gentle inversion three to four times.
7. Enter the PZA set into the BACTEC MGIT 960 instrument using the AST set entry feature (refer to the BACTEC MGIT 960 User's Manual, AST Instructions). 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 BAP. Enclose in a plastic bag. Incubate at 35 - 37°C.
9. Check the BAP at 48 h for bacterial contamination. If the BAP shows no growth, then allow AST testing to proceed. If the BAP shows growth, discard AST set (refer to the BACTEC MGIT 960 User's Manual, AST Instruction) and repeat testing with pure culture

8.0 Interpretation & Reporting of Results

8.1 Susceptible and Resistant Results

The BACTEC MGIT 960 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 instrument.

8.2 Errors

The results are reported 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) are described in the AST Instructions - Troubleshooting of the BACTEC MGIT 960 User's Manual.

8.3 Confirmatory Testing

In the event of unexpected resistant results, verify identification of isolate tested as *M. tuberculosis*. Ensure that only a pure culture was used (rule-out presence of mixed mycobacteria, etc.). Mono-resistance to ethambutol is uncommon and should be verified. Based on the in-house validation of the BACTEC MGIT 960 PZA assay, any PZA resistant isolates should be sent to the WA State lab for confirmation. Review resistant results on Rounds.

8.4 Reporting Results

Each drug name and concentration should be reported with the result of susceptible (S) or resistant (R).

9.0 Quality Control & Quality Assurance

9.1 New Shipment or Lot Number

Upon receipt of a new shipment or lot number of BACTEC MGIT 960 SIRE Kit vials, the control organism should be tested. The control organism should be in pure culture and the culture should be prepared according to the "Inoculum Preparation" instructions.

The quality control (QC) AST set should be prepared according to the "Inoculation Procedure for Susceptibility Test" instruction for the drug kits being tested. Important considerations when

preparing the QC AST Set are the proper reconstitution of the lyophilized drugs and the proper dilution for the Growth Control and drug tubes.

It is important to add the appropriate drug to the corresponding labeled tube. The use of the pan-sensitive QC organism will not detect incorrectly placed drug in the AST Set tubes. Observation of the proper results, as shown below, within 4-13 days indicates that the BACTEC MGIT 960 SIRE Kits are ready for use in testing patient isolates. Observation of the proper results, as shown below, within 4-20 days indicates that the BACTEC MGIT 960 PZA Kits are ready for use in testing patient isolates. If the proper results are not observed, repeat the test. If, after repeating the test, the proper results are still not observed, do not use the product until you have notified the supervisor and have contacted BD Technical Services at (800) 638-8663.

Strain	GC	SIRE	STR 4.0	INH 0.4	PZA
M. tuberculosis ATCC 27294	Positive	Susceptible	Susceptible	Susceptible	Susceptible

9.2 Weekly Quality Control Testing

The same control organism should be run as batch QC once each week when susceptibility testing is performed. If the batch QC fails, do not report patient results for the drug(s) that failed for that testing period. Repeat the QC and patient isolates for the drug(s) affected by the initial QC failure. The most common causes of QC failure are contamination of QC culture, over/under inoculation of AST sets, and drugs added to incorrect tubes or improper placement of tubes. If the repeat QC does not perform as expected, do not report patient results. Notify the supervisor and contact BD Technical Services at (800) 638-8663.

10.0 Limitations

1. The BACTEC MGIT 960 susceptibility test does not interpret the degree of susceptibility of the isolate being tested.
2. The BACTEC MGIT 960 SIRE test was developed with critical concentrations for streptomycin, isoniazid, rifampin, and ethambutol that are slightly lower than the critical concentrations used in the MOP in order to avoid false susceptibility. Testing of the higher concentrations, as recommended, will enhance the ability to detect isolates with low-level resistance.
3. The BACTEC MGIT 960 susceptibility tests can only be performed using the BACTEC MGIT 960 instrument. The AST Sets cannot be read manually.
4. 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.
5. 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 AST Set error.
6. Failure to use the 1:5 dilution of the organism suspension, when indicated, to inoculate the drug containing tubes may give inaccurate results.
7. Failure to use a 1:100 (SIRE) or 1:10 (PZA) dilution of the organism suspension for the inoculation of the Growth Control tube may give inaccurate results or cause an AST Set error.
8. Failure to reconstitute the drugs with the appropriate volume of sterile distilled/deionized water may give inaccurate results.
9. Thorough mixing of inoculated tubes is important. Failure to mix the tubes adequately can lead to false resistant results.
10. 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.
11. Failure to load the AST Set into the instrument correctly will result in an anonymous condition that must be resolved within 8 h. If the condition is not resolved within 8 h, the AST Set must be discarded and set up again.

12. Failure to use SIRE or PZA Supplement in the AST Set may give inaccurate results. DO NOT add BACTEC MGIT 960 Growth Supplement to the AST Set.
13. Failure to use BACTEC MGIT 960 PZA Medium for the PZA AST set may give inaccurate results. Do not substitute with BBL MGIT 7 mL tubes.

11.0 Validation Information

A total of 24 isolates of *Mycobacterium tuberculosis* were used for the validation of the BACTEC MGIT 960 SIRE and PZA susceptibility tests. The test organisms included 6 ATTC strains and 18 clinical isolates. The following ATTC strains were tested: *M. tuberculosis* ATCC 35820, resistant to streptomycin; 35822, resistant to isoniazid; 35838, resistant to rifampin; 35837, resistant to ethambutol; 35828, resistant to pyrazinamide, and 27294, susceptible to all drugs tested. All of the test isolates were previously tested using the BACTEC 460TB radioactive susceptibility method. The SIRE results for all 24 (100%) isolates were the same by both methods. Two (8%) of the 24 test isolates yielded discrepant results for PZA. These two isolates yielded resistant results on the BACTEC MGIT 960 but were susceptible on the BACTEC 460TB. Repeat testing of these isolates did not resolve the discrepancies. A summary of the susceptibility results is shown in the table below.

Susceptibility Results by MGIT 960 and BACTEC 460TB Systems

Drug conc.	Total No. Tested	No. (S) by both methods	No. (R) by both methods	No. that were:		Agreement (%)
				MGIT (R) 460TB (S)	MGIT (S) 460TB (R)	
STR 1.0	24	22	2	0	0	100
INH 0.1	24	21	3	0	0	100
RIF 1.0	24	22	2	0	0	100
EMB 5.0	24	22	2	0	0	100
PZA 100	24	19	3	2	0	91.7

As a result of the PZA discrepancies in this validation study, all future clinical isolates that yield a resistant result will be confirmed by the WA state lab prior to reporting.

Streptomycin resistance was detected at the lower concentration (1.0 μg/mL) in one ATCC strain and one clinical isolate. Further testing of these strains was performed at the higher concentration of 4.0 μg/mL. *M. tuberculosis* ATCC 35820 tested resistant at the higher concentration while the clinical isolate was susceptible. The susceptibility pattern of the clinical isolate correlated with testing performed at the Washington State Department of Health.

Isoniazid resistance was detected at the lower concentration (0.1 μg/mL) in one ATCC strain and in two clinical isolates. Further testing of these strains was performed at the higher concentration of 0.4 μg/mL. *M. tuberculosis* ATCC 35822 and one of the clinical isolates tested resistant at the higher concentration. The other clinical isolate tested susceptible. The susceptibility patterns of both clinical isolates correlated with testing performed at the Washington State Department of Health.

12.0 References

1. Package insert: BD BACTEC™ MGIT™ 960 SIRE Kits, 8008200, 2007/3.
2. Package insert: BD BACTEC™ MGIT™ 960 PZA Kit, L005486JAA, 2007/3.

13.0 Document Control

Adopted/Reviewed by director (AR) and supervisor (JC) 3/2008

Reviewed by Dr. Schappert 3/10/2010

Reviewed by JC 3/2009, 4/2010, 4/20/12, 3/2014

Revisions: 01/08/2013 added the use of N95 respirator to be worn for culture manipulation. Updated document to PPM format with standardized sections.