

BACTEC™ MGIT™ 960 SIRE Kit

For the Antimycobacterial Susceptibility Testing

of *Mycobacterium tuberculosis*

I. INTENDED USE

The **BACTEC™ MGIT™ 960 SIRE Kit** is used as 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 STR 4.0 Kit** and the **BACTEC™ MGIT™ 960 INH 0.4 Kit** are for testing at higher drug concentrations.

The **BACTEC™ MGIT™ 960** susceptibility test kits are used with the **BACTEC™ MGIT™ 960 System**.

II. SUMMARY AND EXPLANATION

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

Multi-drug resistant *Mycobacterium tuberculosis* (MDR – TB) has recently become a serious public health problem.¹ Resistance to any of the primary drugs, streptomycin (STR), isoniazid (INH), rifampin (RIF), and ethambutol (EMB), 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. Resistance to a drug is detected when 1% or more of the bacterial population is resistant to the drug concentration under test. Results are generally available after 21 days of incubation. The second method, known as the **BACTEC™ 460TB** radiometric susceptibility method,³ generally takes from 4 to 12 days. It is based on the production of radioactive ¹⁴C-labeled carbon dioxide by the growing mycobacteria, manifested by a Growth Index increase in the system.

Historically, the Method of Proportion (MOP) procedure has included susceptibility testing of *M. tuberculosis* using two concentrations of antimicrobials. The National Committee for Clinical Laboratory Standards (NCCLS) 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. This result provides information to the physician to assist in determining whether a modification to the therapy regimen is necessary.

The **BACTEC™ MGIT™ 960 SIRE** test provides the susceptibility result in approximately the same time frame as the **BACTEC™ 460TB** system. In addition, this method is non-radiometric and allows appropriate antibiotic susceptibility results to be reported earlier, in most cases, than the MOP procedure.

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. 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, isoniazid and/or ethambutol, should be tested at a higher drug concentration either in the **BACTEC MGIT 960** or using an alternate method. In this case, a final result of resistant at the critical concentration may be reported, with notification that an additional test at a higher concentration is being performed.

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 the MOP. This design of the **BACTEC MGIT 960** system 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.

III. PRINCIPLES OF THE PROCEDURE

The **BBL™ MGIT™ 7 mL Mycobacteria Growth Indicator Tube** is a tube containing a modified Middlebrook 7H9 Broth which supports the growth and detection of mycobacteria (see **BBL MGIT 7 mL** package insert). The **MGIT** 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 **BACTEC MGIT 960 SIRE Kit** is a 4 – 13 day qualitative test. The test is based on growth of the *Mycobacterium tuberculosis* isolate in a drug-containing tube compared to a drug-free tube (Growth Control). The **BACTEC MGIT 960** instrument continuously 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 **BACTEC MGIT 960** instrument automatically interprets these results and reports a susceptible or resistant result.

IV. REAGENTS

BACTEC MGIT 960 SIRE Kit contains one each lyophilized vials of streptomycin, isoniazid, rifampin and ethambutol, and eight vials of SIRE Supplement.

Approximate Formula* Per Vial Lyophilized drug: Streptomycin332 µg.
Approximate Formula* Per Vial Lyophilized drug: Isoniazid33.2 µg.
Approximate Formula* Per Vial Lyophilized drug: Rifampin.....332 µg.
Approximate Formula* Per Vial Lyophilized drug: Ethambutol1660 µg.

BACTEC MGIT STR 4.0 Kit contains one vial lyophilized streptomycin and two vials of SIRE Supplement.

Approximate Formula* Per Vial Lyophilized drug: Streptomycin.....664 µg.

BACTEC MGIT INH 0.4 Kit contains one vial lyophilized isoniazid and two vials of SIRE Supplement.

Approximate Formula* Per Vial Lyophilized drug: Isoniazid66.4 µg.

BACTEC MGIT 960 SIRE Supplement contains 20 mL Middlebrook OADC enrichment

Approximate Formula* Per L Purified Water

Bovine albumin	50.0 g	Catalase	0.03 g
Dextrose	20.0 g	Oleic Acid	0.6 g

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

Storage and reconstitution of reagents: BACTEC MGIT 960 SIRE Drug vials – On receipt, store the lyophilized drug vials at 2 - 8° C. 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 immediately. Discard unused portions.

BACTEC MGIT SIRE Supplement – On receipt, store in 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 **BACTEC MGIT 960 SIRE Kit Streptomycin** lyophilized drug vial with **4 mL** of sterile distilled/deionized water to make a stock solution of 83 µg/mL.

Reconstitute each **BACTEC MGIT 960 SIRE Kit Isoniazid** lyophilized drug vial with **4 mL** of sterile distilled/deionized water to make a stock solution of 8.3 µg/mL.

Reconstitute each **BACTEC MGIT 960 SIRE Kit Rifampin** lyophilized drug vial with **4 mL** of sterile distilled/deionized water to make a stock solution of 83 µg/mL.

Reconstitute each **BACTEC MGIT** 960 SIRE Kit Ethambutol lyophilized drug vial with **4 mL** of sterile distilled/deionized water to make a stock solution of 415 µg/mL.

NOTE: The following are reconstituted with a different volume. Failure to use the appropriate volume of sterile distilled water for reconstitution of the higher drug concentrations will invalidate those test results.

Reconstitute each **BACTEC™ MGIT™** 960 STR 4.0 Kit Streptomycin lyophilized drug vial with **2 mL** of sterile distilled/deionized water to make a stock solution of 332 µg/mL.

Reconstitute each **BACTEC™ MGIT™** 960 INH 0.4 Kit Isoniazid lyophilized drug vial with **2 mL** of sterile distilled/deionized water to make a stock solution of 33.2 µg/mL.

V. WARNINGS:

For *in vitro* Diagnostic Use.

POTENTIALLY INFECTIOUS TEST SPECIMEN: Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. “Standard Precautions”⁴⁻⁷ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.

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 **BBL™ MGIT™** 7 mL Mycobacteria Growth Indicator Tube.

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 **MGIT** tubes prior to disposal.

VI. SPECIMEN PREPARATION

All preparations detailed below are 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*.

Preparation of the Isolate 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 **BBL™**Middlebrook 7H9 Broth (or **BBL 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 14 days 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 in 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 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 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 suspension to a 0.5 McFarland standard by a 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).

Preparation from a Positive BACTEC MGIT 7 mL Tube:

NOTE: It is important to prepare the inoculum using the following time references to obtain the appropriate organism concentration for the susceptibility test.

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 five 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.
3. If the tube is a Day 1 or Day 2 positive, mix well and 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 positive broth in 4 mL of sterile saline (1:5 dilution). Use the diluted suspension for the inoculation procedures. Proceed to “Inoculation Procedure for Susceptibility Test.”

VII. PROCEDURE

Materials Provided: **BACTEC MGIT 960 SIRE Kit** containing one vial each lyophilized drug and eight vials of SIRE Supplement (approximately 40 tests per drug per kit). **BACTEC MGIT 960 STR 4.0 Kit** containing one vial each lyophilized drug and two vials of SIRE Supplement (approximately 20 tests per kit) and **BACTEC MGIT 960 INH 0.4 Kit** containing one vial each lyophilized drug and two vials of SIRE Supplement (approximately 20 tests per kit).

Materials Required But Not Provided: **BBL MGIT 7 mL Mycobacteria Growth Indicator Tubes**, ancillary culture media, reagents, quality control organisms and laboratory equipment as required for this procedure.

Inoculation Procedure for BACTEC MGIT 960 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 (see **BACTEC MGIT 960 User's Manual**, AST Instructions).
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 pipet, using a micropipet, 100 µL of 83 µg/mL **MGIT STR** solution to the appropriately labeled **MGIT** tube. Aseptically pipet 100 µL of 8.3 µg/mL **MGIT INH** solution to the appropriately labeled **MGIT** tube. Aseptically pipet 100 µL of 83 µg/mL **MGIT RIF** solution to the appropriately labeled **MGIT** tube. Aseptically pipet 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	100 µL	1.0 µg/mL
MGIT EMB	415 µg/mL	100 µL	5.0 µg/mL

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

4. **Growth Control tube preparation and inoculation:** Aseptically pipet 0.1 mL of the organism suspension (see "Specimen 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 pipet 0.5 mL of the organism suspension (see "Specimen Preparation") into each of the FOUR remaining drug tubes (STR, INH, RIF, EMB).

6. Tightly recap the tubes. Mix tubes thoroughly by gentle inversion 3 to 4 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 **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 h for bacterial contamination. If the blood agar plate shows no growth, then allow AST testing to proceed. If the blood agar plate shows growth, discard the AST set (refer to the **BACTEC MGIT 960 User's Manual**, AST Instructions) and repeat testing with pure culture.

Inoculation Procedure for BACTEC MGIT STR 4.0 and INH 0.4 Kits 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 "Specimen 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 under "Specimen Preparation: Preparation from a Positive **MGIT** tube."

1. Label enough **MGIT 7 mL** tubes for the test isolate to have a **MGIT GC** (Growth Control) and a **MGIT** drug tube for each antimicrobial tested. Place the tubes in the correct sequence in the appropriate size AST Set Carrier (see **BACTEC MGIT 960 User's Manual**, AST Instructions).
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 pipet, using a micropipet, 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 **2 mL** sterile distilled/deionized water to achieve concentrations indicated.

4. **Growth Control tube preparation and inoculation:** Aseptically pipet 0.1 mL of the organism suspension (see “Specimen 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 pipet 0.5 mL of the organism suspension (see “Specimen 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 **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 h for bacterial contamination. If the blood agar plate shows no growth, then allow AST testing to proceed. If the blood agar plate shows growth, discard the AST set (refer to the **BACTEC MGIT 960 User’s Manual, AST Instructions**) 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 **BACTEC MGIT 960 User’s Manual, AST Instructions**).

VIII. QUALITY CONTROL

User Quality Control: Upon receipt of a new shipment or lot number of **BACTEC MGIT 960 SIRE Kit** vials, 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 the “SPECIMEN PREPARATION” instructions.

The quality control (QC) AST Set should be prepared according to the “Inoculation Procedure for Susceptibility Test” instructions 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 of the QC organism 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 incorrect drug pipetted into 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. 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 contacted Technical Services at (800) 638-8663 (United States only).

Strain	GC	MGIT STR	MGIT INH	MGIT RIF	MGIT EMB
<i>M.tuberculosis</i> ATCC™ 27294	Positive	Susceptible	Susceptible	Susceptible	Susceptible

Strain	GC	MGIT STR 4.0	MGIT INH 0.4
<i>M.tuberculosis</i> ATCC™ 27294	Positive	Susceptible	Susceptible

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 for the drug(s) and 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 (800) 638-8663 (United States only).

During the external evaluation of the **BACTEC MGIT 960 SIRE Kits**, the most common causes of QC failure were contaminated QC cultures, over/under inoculated AST Sets, drug not added to appropriate tubes and instrument error conditions.

IX. REPORTING OF 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 **BACTEC MGIT 960** instrument (refer to the **BACTEC MGIT 960 User's Manual**, AST Instructions). The **BACTEC MGIT 960** 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 the AST Instructions, Section 7- Troubleshooting of the **BACTEC MGIT 960 User's Manual**.

When reporting results, it is important to include the test method, drug name and concentration, whether the result is obtained with the **BACTEC MGIT 960** or an alternate method. The Pulmonary and/or Infectious Disease specialist in TB control should be consulted concerning the appropriate therapeutic regimen and dosages.

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.^{2,8}

BACTEC MGIT 960 SIRE critical concentration result reporting

Drug (concentration)	MGIT 960 result	Recommended Report	Action
STR (1.0 µg/mL)	Susceptible (SIRE)	Isolate tested with BACTEC MGIT 960 [drug/concentration] and result is susceptible.	No action.
INH (0.1 µg/mL) RIF (1.0 µg/mL) EMB (5.0 µg/mL)	Resistant (SIR)	Isolate tested with BACTEC MGIT 960 [drug/concentration] and result is resistant. Results of testing [drug] at a higher concentration to follow (if tested).	Recommend testing at higher concentration (STR and/or INH).
	Resistant (E)	<u>If resistant to more than ethambutol (EMB)</u> Isolate tested with BACTEC MGIT 960 Ethambutol 5.0 µg/mL and result is resistant. Consult laboratory for testing EMB at a higher concentration. <u>If mono-resistant to ethambutol (EMB)</u> Isolate tested with BACTEC MGIT 960 Ethambutol 5.0 µg/mL and result is resistant. Mono-resistance to ethambutol is uncommon. Consult laboratory for confirmation.	Recommend testing EMB at higher concentration using alternate method. Recommend testing EMB at both critical concentration and higher concentration with an alternate method.
	Error (X)	No report.	Repeat test.

BACTEC MGIT 960 STR 4.0 and INH 0.4 result reporting

Drug (concentration)	MGIT 960 result	Recommended Report	Action
STR (4.0 µg/mL)	Susceptible	Isolate tested with BACTEC MGIT 960 streptomycin 4.0 µg/mL and result is susceptible. This isolate, with a resistant result at 1.0 µg/mL and a susceptible result at 4.0 µg/mL, indicates low-level resistance to streptomycin.	No action
	Resistant	Isolate tested with BACTEC MGIT 960 streptomycin 4.0 µg/mL and result is resistant. Consult laboratory for confirmation.	Isolate should be tested by an alternate method to verify result.
	Error (X)	No report.	Repeat test
INH (0.4 µg/mL)	Susceptible	Isolate tested with BACTEC MGIT 960 isoniazid 0.4 µg/mL and result is susceptible. This isolate, with a resistant result at 0.1 µg/mL and a susceptible result at 0.4 µg/mL, indicates low-level resistance to isoniazid.	No action
	Resistant	Isolate tested with BACTEC MGIT 960 isoniazid 0.4 µg/mL and result is resistant.	No action
	Error (X)	No report.	Repeat test

X. LIMITATIONS OF THE PROCEDURE

The **BACTEC MGIT 960** susceptibility test does not interpret the degree of susceptibility of the isolate being tested. Results are reported as either S, susceptible, or R, resistant, for the drug and concentration tested.

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.

The **BACTEC MGIT 960** susceptibility tests can only be performed using the **BACTEC MGIT 960** instrument. The AST 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 1:100 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 drugs 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 can 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 eight hours. If condition is not resolved within eight hours, the AST Set must be discarded and set up again.

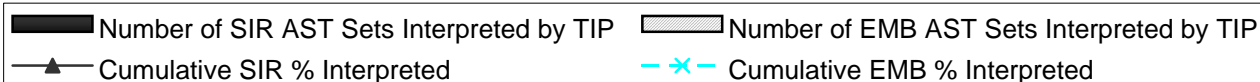
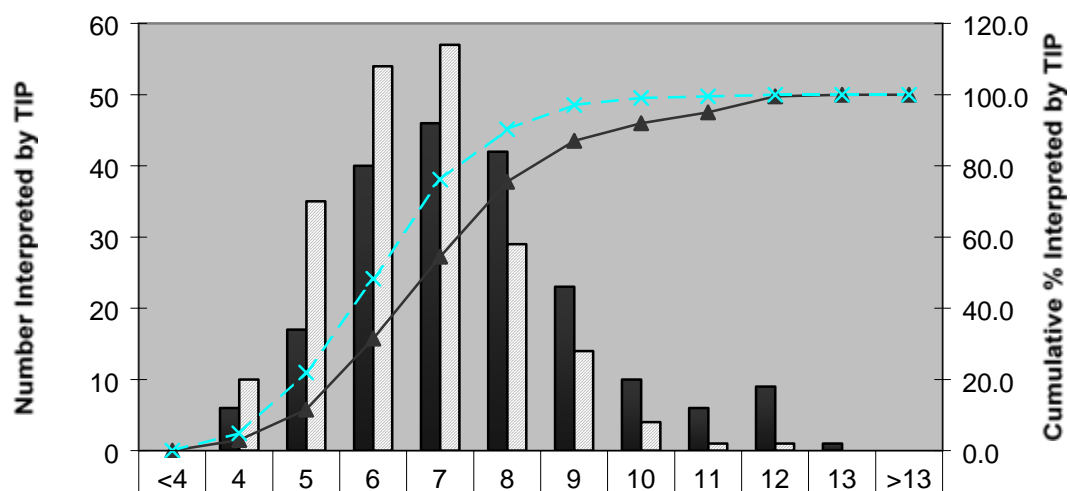
Failure to use the SIRE Supplement in the AST Set may give inaccurate results. **DO NOT** add **BACTEC MGIT 960** Growth Supplement to the AST Set.

XI. EXPECTED RESULTS

A total of 106 clinical isolates of *M. tuberculosis* were tested with the **BACTEC MGIT 960** SIRE susceptibility test at four geographically diverse sites. The testing included both fresh clinical and stock isolates from both liquid and solid culture sources. A total of 200 susceptibility tests (liquid and solid) were performed at the critical concentrations of streptomycin (STR), isoniazid (INH) and rifampin (RIF) and a total of 223 susceptibility tests (liquid and solid) were performed at the critical concentration of ethambutol (EMB) during separate testing. The overall average time-to-result for the **BACTEC MGIT 960** SIRE susceptibility test is seven to eight days with a range from four to fourteen days. The data are shown in Figure 1.

Figure 1: Distribution of BACTEC MGIT 960 AST Time in Protocol

Frequency Distribution of Time in Protocol (TIP)



XII. PERFORMANCE CHARACTERISTICS

Analytical Studies

Liquid and Solid Media AST Inoculum Ranges:

*Liquid media*_- The recommended procedure for preparing an AST Set from a positive **MGIT** 7 mL tube 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 0.8×10^5 to 3.2×10^5 CFU/mL.

*Solid media*_- The recommended procedure for preparing an AST Set from growth on solid media (up to 14 days) 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 1.4×10^5 to 2.4×10^6 CFU/mL.

Lot Reproducibility:

Lot reproducibility was evaluated using twenty-five *M. tuberculosis* isolates (to include five ATCC™ strains). Each **BACTEC MGIT 960 SIRE** test at the critical drug concentration was performed in triplicate for a total of seventy-five results per drug. Each replicate represented a separate test condition differentiated by lot of SIRE drug and SIRE Supplement used (three lots each).

Those isolates that were determined resistant to streptomycin, isoniazid or ethambutol in the initial test were then tested with the high drug concentration, except the ATCC™ strains. In addition to the resistant isolates tested, two sensitive isolates to STR (critical concentration), two sensitive isolates to INH (critical concentration) and two sensitive isolates to EMB (critical concentration) were included in the susceptibility profile test. Observed results were compared to the expected results.

The overall reproducibility for each drug at the critical concentration is 96% for STR, 100% for INH, 100% for RIF and 100% for EMB. The overall reproducibility for each drug at the high concentration is 96% for STR 4.0 and 100% for INH 0.4.

CDC Challenge Panel Testing:

The performance of the **BACTEC MGIT 960 SIRE** susceptibility test was evaluated using a panel of challenge isolates obtained from the Centers for Disease Control and Prevention (CDC). The panel consisted of thirty isolates of *M. tuberculosis* with known susceptibility patterns (using MOP). The panel was tested twice with the **BACTEC MGIT 960 SIRE** susceptibility test and both results were in agreement. The **BACTEC MGIT 960 SIRE** results were compared to the CDC expected results.

The overall agreement with CDC expected results for each drug at the critical concentration is 93% for STR, 100% for INH, 100% for RIF and 100% for EMB. The overall agreement with CDC expected results for each drug at the high concentration is 100% for STR 4.0 and 100% for INH 0.4.

Clinical Evaluation

The **BACTEC MGIT 960 SIRE** susceptibility test was evaluated at four geographically diverse clinical sites, composed of regional reference centers and university hospital-based laboratories, including one foreign site. The **BACTEC MGIT 960 SIRE** susceptibility test was compared to the Method of Proportion (MOP)² susceptibility test method. The initial evaluation included the drugs streptomycin, isoniazid and rifampin. A separate evaluation was performed for the drug ethambutol.

Reproducibility Testing:

The reproducibility of the **BACTEC MGIT 960 SIRE** test was evaluated at the clinical sites using a panel of ten qualified isolates, including several isolates resistant to each of the drugs. The **BACTEC MGIT 960 SIRE** test results were compared to the expected results. The overall reproducibility for each drug at the critical concentration is 98.9% for STR, 99.7% for INH, 99.2% for RIF and 97.5% for EMB. Individual site reproducibility ranged from 89.9% to 100% for the combined critical concentration drug results. The overall reproducibility for each drug at the high concentration is 99.7% for STR 4.0 and 95.6% for INH 0.4. Individual site reproducibility ranged from 92.2% to 100% for the combined high concentration drug results.

CDC Challenge Panel Testing:

The performance of the **BACTEC MGIT 960 SIRE** susceptibility test was evaluated using a panel of challenge isolates obtained from the Centers for Disease Control and Prevention (CDC). The panel consisted of thirty isolates of *M. tuberculosis* with known susceptibility patterns (using MOP) tested by each clinical site.

Table 1 shows the agreement of the **BACTEC MGIT 960 SIRE** susceptibility test for each drug compared to the CDC expected results.

Table 1: CDC Challenge Panel – BACTEC MGIT 960 Clinical Site Testing

MGIT 960	Number tested	Number Correct	% Correct
STR 1.0	120	111	92.5
INH 0.1	120	119	99.2
RIF 1.0	120	120	100
EMB 5.0	119	111	93.3
STR 4.0	29*	29	100
INH 0.4	87*	82	94.3

*Only isolates resistant at critical concentrations tested at STR 4.0 and INH 0.4.

Clinical Isolate Testing:

A total of 106 clinical isolates of *M. tuberculosis* were tested with the **BACTEC MGIT 960** SIRE susceptibility test and the MOP susceptibility test. This included testing of both fresh clinical and stock isolates from both liquid and solid culture sources. This generated a total of 195 test results for the initial susceptibility test performed for streptomycin, isoniazid and rifampin (critical concentration). A separate evaluation of ethambutol was performed from frozen aliquots of the original clinical and stock isolates as well as prospective clinical isolates from both liquid and solid culture sources. This generated a total of 223 test results for the ethambutol test at the critical concentration.

Table 2 presents the results from clinical isolate testing for each drug (critical concentration) from liquid source cultures. Table 3 presents the results from clinical isolate testing for each drug (critical concentration) from solid source cultures.

Table 2: Clinical Isolate Results – BACTEC MGIT 960 AST Compared to Method of Proportion from Liquid Source Cultures

DRUG	Method of Proportion			MGIT 960 AST System				
	Concentration	S	R	Concentration	Susceptible Results		Resistant Results	
					# Agreement	% Agreement (95% CI)	# Agreement	% Agreement (95% CI)
STR	2.0 µg/mL	69	27	1.0 µg/mL	62	90 (80– 96)	26	96 (81–100)
INH	0.2 µg/mL	59	37	0.1 µg/mL	57	97 (88-100)	36	97 (86-100)
RIF	1.0 µg/mL	72	24	1.0 µg/mL	71	99 (93-100)	24	100 (95-100)
EMB	5.0 µg/mL	91	20	5.0 µg/mL	88	97 (91 – 99)	17	85 (62 – 97)

All isolates with discordant **MGIT** results were tested by MOP at two independent sites. Of the seven discordant STR resistant (R-960, S-MOP) isolates, three had resistant results from both sites and one had susceptible results from both sites. The remaining three had resistant results from one site and susceptible results from the other site. The discordant STR susceptible (S-960, R-MOP) isolate had susceptible results from both sites. The two discordant INH resistant (R-960, S-MOP) isolates had susceptible results from both sites. The discordant INH susceptible (S-960, R-MOP) isolate had susceptible results from both sites. The discordant RIF resistant (R-960, S-MOP) isolate had resistant results from both sites. The three discordant EMB resistant (R-960, S-MOP) isolates had susceptible results from both sites. Of the three discordant EMB susceptible (S-960, R-MOP) isolates, two had susceptible results from both sites and one had a resistant result from one site and a susceptible result from the other site.

Table 3: Clinical Isolate Results – BACTEC MGIT 960 AST Compared to Method of Proportion from Solid Source Cultures

DRUG	Method of Proportion			MGIT 960 AST System				
	Concentration	S	R	Concentration	Susceptible Results		Resistant Results	
					# Agreement	% Agreement (95% CI)	# Agreement	% Agreement (95% CI)
STR	2.0 µg/mL	70	29	1.0 µg/mL	65	93 (84-98)	28	97 (82-100)
INH	0.2 µg/mL	63	36	0.1 µg/mL	62	98 (92-100)	35	97 (86-100)
RIF	1.0 µg/mL	70	29	1.0 µg/mL	70	100 (95-100)	26	90 (73-98)
EMB	5.0 µg/mL	87	25	5.0 µg/mL	86	99 (94-100)	20	80 (59-93)

All isolates with discordant **MGIT** results were tested by MOP at two independent sites. Of the five discordant STR resistant (R-960, S-MOP) isolates, two had resistant results from both sites and one had susceptible results from both sites. The remaining two had resistant results from one site and susceptible results from the other site. The discordant STR susceptible (S-960, R-MOP) isolate had resistant results from both sites. The discordant INH resistant (R-960, S-MOP) isolate had susceptible results from both sites. The discordant INH susceptible (S-960, R-MOP) isolate had resistant results from both sites. The three discordant RIF susceptible (S-960, R-MOP) isolates had susceptible results from both sites. The one discordant EMB resistant (R-960, S-MOP) isolate had resistant results from both sites. Of the five discordant EMB susceptible (S-960, R-MOP) isolates, four had susceptible results from both sites. The remaining isolate had a resistant result from one site and a susceptible result from the other site.

Table 4 presents the results from clinical isolate testing for streptomycin and isoniazid (high concentration) from liquid source cultures. Table 5 presents the results from clinical isolate testing for streptomycin and isoniazid (high concentration) from solid source cultures.

Table 4: Clinical Isolate Results – BACTEC MGIT 960 AST Compared to Method of Proportion from Liquid Source Cultures

DRUG	Method of Proportion			MGIT 960 AST System				
	Concentration	S	R	Concentration	Susceptible Results		Resistant Results	
					# Agreement	% Agreement (95% CI)	# Agreement	% Agreement (95% CI)
STR	10.0 µg/mL	77	19	4.0 µg/mL	73*	95 (87-99)	17	90 (67-99)
INH	1.0 µg/mL	65	31	0.4 µg/mL	65*	100 (95-100)	29	94 (79-99)

* Assumes MGIT high drug S result for all isolates with MGIT low drug S result.

All isolates with discordant **MGIT** results were tested by MOP at two independent sites. The four discordant STR resistant (R-960, S-MOP) isolates had susceptible results from both sites. Of the two discordant STR susceptible (S-960, R-MOP) isolates, one had susceptible results from both sites and one had resistant results from both sites. Of the two discordant INH susceptible (S-960, R-MOP) isolates, one had susceptible results from both sites and one had resistant results from both sites.

Table 5: Clinical Isolate Results – BACTEC MGIT 960 AST Compared to Method of Proportion from Solid Source Cultures

DRUG	Method of Proportion			MGIT 960 AST System				
	Concentration	S	R	Concentration	Susceptible Results		Resistant Results	
					# Agreement	% Agreement (95% CI)	# Agreement	% Agreement (95% CI)
STR	10.0 µg/mL	78	21	4.0 µg/mL	73*	94 (86-98)	17	81 (58-95)
INH	1.0 µg/mL	68	31	0.4 µg/mL	68*	100 (95-100)	30	97 (83-100)

* Assumes MGIT high drug S result for all isolates with MGIT low drug S result.

All isolates with discordant **MGIT** results were tested by MOP at two independent sites. The five discordant STR resistant (R-960, S-MOP) isolates had susceptible results from both sites. Of the four discordant STR susceptible (S-960, R-MOP) isolates, three had susceptible results from both sites and one had resistant results from both sites. The discordant INH susceptible (S-960, R-MOP) isolate had resistant results from both sites.

XIII. AVAILABILITY

Cat. No.	Description	Cat. No.	Description
245123	BACTEC™ MGIT™ 960 SIRE Kit, carton of 4 lyophilized drug vials, and 8 SIRE Supplements.	245126	BACTEC™ MGIT™ 960 INH 0.4 Kit, carton of 1 lyophilized drug vial and 2 SIRE Supplements.
245125	BACTEC™ MGIT™ 960 STR 4.0 Kit, carton of 1 lyophilized drug vial and 2 SIRE Supplements.		

XIV. REFERENCES

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