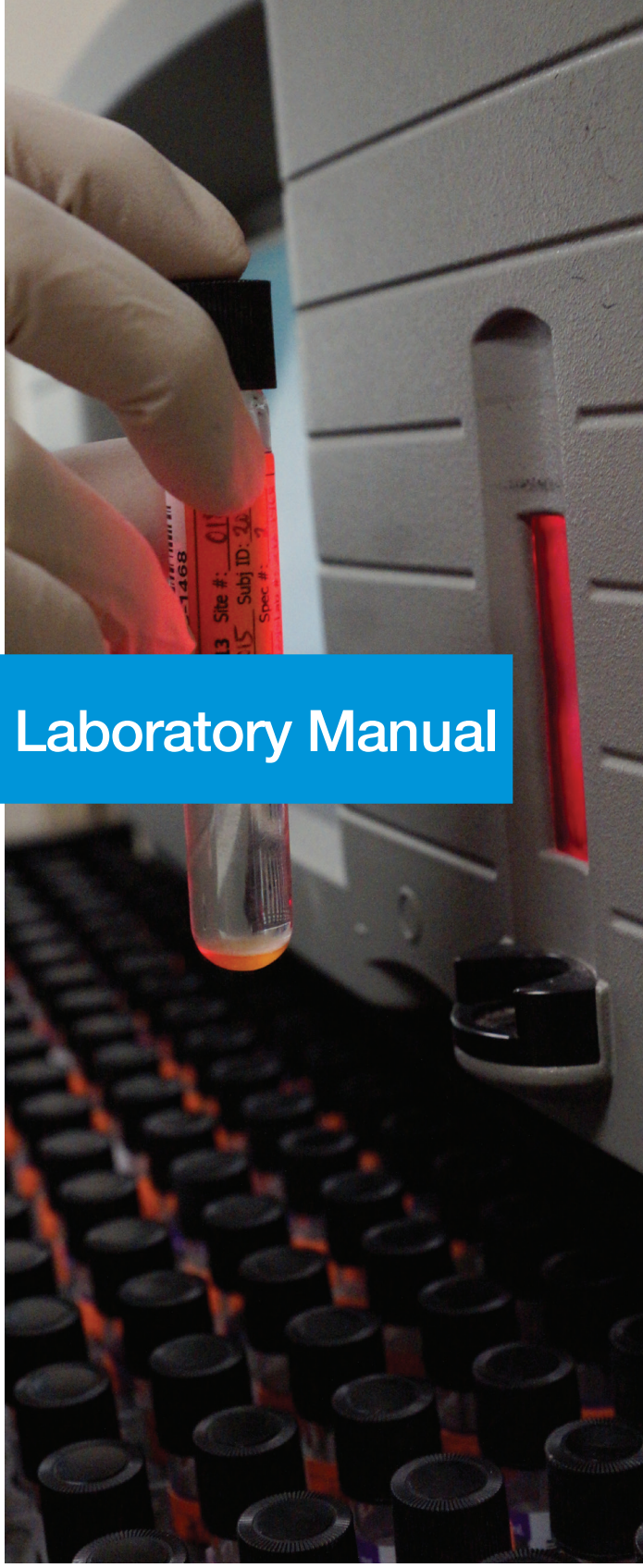
global laboratory initiative
advancing TB diagnosis



Mycobacteriology Laboratory Manual

First Edition, April 2014

A publication of the Global Laboratory Initiative
a Working Group of the Stop TB Partnership

Stop TB Partnership

This document was developed to ensure high quality results and comparability of data from a network of international tuberculosis laboratories handling sputum specimens for Otsuka Pharmaceutical Development and Commercialization (OPDC)-sponsored multidrug-resistant tuberculosis (MDR-TB) clinical trials for the development of Deltyba® (delamanid). It consists of standardized procedures related to sputum collection, handling, analyses, and reporting, with a focus on testing that has the greatest impact on microbiology endpoints for MDR-TB clinical trials (sputum culture conversion in liquid and solid media).

A specialized Otsuka team, including a microbiology expert with extensive experience in mycobacteriology laboratory-based research in international settings, a senior clinical research associate (CRA) with prior experience in international TB research, and a senior laboratory technologist with clinical experience working in several international TB laboratories, drafted the first version of this manual. Input from multiple lab directors/managers and consultants in the Otsuka trials network, as well as review of data generated from the clinical trials themselves, prompted several revisions incorporated in this current version of the document.

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LIST OF ABBREVIATIONS AND ACRONYMS

AFB	Acid fast bacilli
AST	Antimycobacterial susceptibility testing; see DST
BAP	Blood agar plate
BD	Becton Dickinson
BSC	Biological Safety Cabinet
BSL	Biosafety level
COA	Certificate of analysis
CDC	US Centers for Disease Control and Prevention
CFR	US Code of Federal Regulations
CFU	Colony forming unit
CLSI	Clinical Laboratory Standards Institute
CRA	Clinical Research Associate
CRC	Clinical Research Coordinator
DST	Drug susceptibility testing; see AST
EMB	Ethambutol
FIND	Foundation for Innovative New Diagnostics
GC	Growth control
GU	Growth unit
ICA	Immunochromatographic assay
INH	Isoniazid
IUATLD	International Union Against Tuberculosis and Lung Disease
LJ	Löwenstein-Jensen
MDR-TB	Multidrug resistant tuberculosis
MTB	*Mycobacterium tuberculosis complex*
MGIT	Mycobacteria Growth Indicator Tube
MOTT	Mycobacteria other than TB
NALC	N-acetyl-L-cysteine
NaOH	Sodium Hydroxide
NTF	Note to File
NTM	Nontuberculous mycobacteria
PANTA	Antibiotic supplement for MGIT tubes

PCR Polymerase chain reaction

PPE Personal protective equipment

PZA Pyrazinamide

OADC Oleic acid, albumin, dextrose, and catalase

QA Quality Assurance

QC Quality Control

QI Quality Improvement

QM Quality Management

RIF Rifampicin

SIRE Streptomycin, isoniazid, rifampicin, ethambutol

SOP Standard Operating Procedure

STR Streptomycin

TB Tuberculosis

TIP Time in protocol (from the MGIT printout)

TNTC Too numerous to count

TSA Trypticase Soy Agar

TTD Time to detection

UV Ultraviolet

WHO World Health Organization

ZN Ziehl-Neelsen stain

1 INTRODUCTION

The diagnosis, treatment, and monitoring of tuberculosis are conducted in a wide range of laboratory facilities worldwide, using a variety of methods, equipment, and capacity. Clinical trials for new tuberculosis drugs base efficacy on microbiologic endpoints, such as sputum culture conversion (SCC) of *Mycobacterium tuberculosis* (MTB). To ensure high quality results and comparability of data from all participating laboratories, a comprehensive laboratory manual that standardizes key laboratory procedures was developed and implemented for a multi-country, randomized, placebo-controlled, Phase II for clinical trials for the treatment of multidrug-resistant tuberculosis (MDR-TB).

The laboratory manual references best practices from the Clinical and Laboratory Standards Institute (CLSI), the Foundation for Innovative New Diagnostics (FIND) *MGIT Procedure Manual,* and laboratory procedures from the National Institutes of Health TB Research Unit, as well as biosafety recommendations from the World Health Organization (WHO) and the US Centers for Disease Control and Prevention (CDC). Each procedure lists the materials, equipment, and forms required for the test, along with the applicable quality control procedures.

The underlying goal of the laboratory manual is to maximize recovery of MTB, even in the presence of contaminants, to provide an accurate picture of the microbiological performance of the investigational compound. Thus, recovery efforts for MGIT cultures are highlighted in several flow charts designed to address scenarios such as mixed cultures, contamination, and early positive cultures (positive MGIT signal with negative confirmatory tests).

In addition, quality and standardized reporting is highlighted throughout the manual to ensure consistent reporting across laboratories. Finally, as clinical trials are highly regulated, quality control (QC) and quality assurance (QA) are of utmost importance in the laboratory. The manual provides detailed QA/QC procedures, as well as comprehensive appendices to record these data.

1.1 Study Data: Maintenance of Documents

1.1.1 Source Documents

All information recorded for the purposes of the study, regardless of form or the media on which it may be recorded, is considered 'source data'. Source data are contained in 'source documents', i.e., the original records where results and observations are entered.

Items that constitute source documents include, but are not limited to:

- Manually-collected data such as lab requisitions, lab registers, and laboratory worksheets (AFB smear log, DST worksheets, QC records, etc.)
- Data generated from automated instruments; e.g., MGIT unloaded positive, negative, and DST reports
- Specimen Transfer Form, Appendix A
- Study Source Document, Appendix B
- QA/QC records

All original source documents must be maintained; do not destroy or discard these records.

1.1.2 Notes to Files

A Note to File (NTF) is a document used to describe an error (i.e. protocol/lab manual deviation) or provide clarification for a result. The Sponsor (an individual, institution, company or organization

that takes the responsibility to initiate, manage or finance a clinical trial) must have a standard template with the following three sections:

- Description of the issue(s)
- Steps taken to mitigate the issue(s)
- Resolution of the issue(s)

NTFs must be filed in the study lab binder.

1.1.3 Appendix K Forms

An Appendix K form is required when a QC test fails to give proper results. It is also used to document any quality improvements made by the laboratory. See Section 16.5: Quality Improvement for an example of how to complete this form. In some cases, a situation may require both an Appendix K and a NTF. If there is any uncertainty about which form to complete, discuss with the lab director and/or the Sponsor.

1.1.4 Document Filing and Access

The timely filing of essential documents in the laboratory, using an organized system of indexing and labeling, greatly assists in the successful management of the study.

Since laboratory documents are subject to review by CRAs, auditors, and regulatory inspectors, they must be stored in a manner that facilitates easy retrieval and review, while protecting the confidentiality of all patients (study and non-study).

For those laboratories using computerized systems that are **not** 21 CFR Part 11 compliant to capture and retain source documents electronically, the following procedure must be followed:

- The lab director or designee must verify (in writing) the data that the system captures (including location/documentation of each data point) and how the system is controlled (e.g., only authorized individuals have access to the system and an audit trail is maintained). If quality control records are stored electronically, these data must also be printed and stored appropriately.
- The lab must ensure that all data for each subject are printed out and filed, by patient and visit interval, in the lab binder. Paper printouts must identify the screening and/or subject ID number, date of printout, and clear page numbering.
- Each page of the printout must be initialed and dated by the lab director or designee. The following information must be captured on the last page of the printout either written in ink or by means of a pre-printed label:
  – Total number of pages in the printout
  – Date and signature of the lab director or designee
  – A statement that reads: 'I confirm that the contents of this printout fully reflect the data captured for this subject as of today'.
- Each time modifications are made to data that has previously been printed, the appropriate page/section must be printed again and the lab director or designee must initial and date the change. The previous printout must remain in the laboratory binder (**do not discard**). The lab director or designee must also inform the site coordinator, data entry person, and site monitor of the modifications. This notification should be documented by the appropriate laboratory staff.

The Microbiology Lab Worksheets binder provided by the Sponsor must contain at least seven tabs, listed below. If additional binders are required, please notify your CRA.

Appendix N - Laboratory Visitor Log

- Each time someone visits the lab to discuss a trial, s/he must sign the visitor log (Appendix N) with the date and purpose of the visit. The laboratory director or delegate must countersign for each visitor.
- Visitors include, but are not limited to: auditors, CRAs, Sponsor representatives, and site staff.

Appendix A - Specimen Transfer Forms

- File the original form in the laboratory binder(s) under the "Specimen Transfer Form" tab by Subject ID and visit number.
- Upon completion of each form, copy and send it to the Clinical Research Coordinator (CRC) for inclusion in the subject's binder.
- Insert additional 'tabs' to separate patients by Subject IDs, especially when the lab receives a large volume of specimens.
- Alternatively, file the Appendix A form in front of the appropriate Appendix B form according to subject ID and visit interval.

Appendix B - Study Source Document Worksheets

- File the original form in the laboratory binder(s) consecutively by Subject ID, and sequentially by visit interval.
- Insert 'tabs' to separate patients by Subject IDs, especially when the lab receives a large volume of specimens.
- Update information from lab source registers and worksheets onto the form in a timely manner.
- Remove the form from the binder only when necessary to make a copy with updated material for the CRC; the technician or supervisor must sign their initials and the date in the margin next to new information that is being sent to the site.
- Supervisor must sign and date the form in the designated spaces when all tests for the specimen have been completed, to signify that the data has been reviewed and is accurate.
- MGIT printouts and AST Reports can also be filed in this section, behind the corresponding Appendix B form.

QC Forms

- If the lab has a separate filing system for these documents, it is acceptable to maintain that system. However, either a note-to-file explaining the location of these forms should be placed under the "QC Forms" tab of the binder, or a copy of the forms containing information related to study specimens should be filed under the "QC Forms" tab of the binder, particularly at the conclusion of the trial.
- Examples of documents that may be filed in the binder include, but are not limited to:
    - Media QC forms
    - Equipment maintenance records
    - Proficiency testing results
    - Monthly data monitoring forms/worksheets

Equipment Temperature Logs

- If the lab has a separate filing system for these logs, it is acceptable to maintain that system. However, either a note-to-file explaining the location of these forms should be placed under the "Equipment Temperature Logs" tab of the binder, or a copy of the forms used during the time period of the study should be filed under the "Equipment Temperature Logs" tab of the binder, particularly at the conclusion of the trial.

Shipping Records

- All records for the shipment of specimens to the central laboratory must be maintained in this section, including copies of requisition forms and airway bills.

Trial Correspondence

- All trial-related correspondence, including emails and faxes, should be maintained in this section.

1.1.5 Document Storage

Records must be kept in a secure location that maintains their integrity and confidentiality, such as a locked room or file cabinet with access restricted to authorized personnel. Storage conditions must also minimize the risk of environmental damage and the threat of accidental destruction, such as from fire or flood. Confidentiality of both study patients and non-study patients must be maintained at all times.

1.2 Study Data: Retention of Documents

The retention of all original study data shall be the responsibility of the Principal Investigator (the individual responsible for conducting the clinical trial and who assumes accountability for the treatment of human subjects and the integrity of the trial data) on behalf of the Sponsor, but at all times shall remain the property of the Sponsor.

Laboratory study records, including all source documents as described in Section 1.1, must be retained and be made available for review under appropriate circumstances, until permission for their disposal has been given by the Sponsor.

Records will be stored in the laboratory's facilities unless permission to transfer and store elsewhere is agreed upon between the laboratory and the Sponsor.

1.3 Supervision of the Study

A supervisor must be assigned to oversee the trial laboratory staff and procedures to ensure the strict adherence to this laboratory manual. At a minimum, this person is responsible for the following oversight activities:

- Ensuring relevant staff are trained on the manual guidelines and procedures, and have read, understood, and will follow the laboratory manual
- Routine and timely review of Appendix B to verify completion and accuracy of all tests
- Monthly review of quality control, quality monitoring, and quality improvement activities, including signing of all QC forms/worksheets and confirmation of test results
- Semi-annual review and follow-up of study-related proficiency testing results
- Investigation of unacceptable or out of range QC results, and proper documentation of corrective actions and improvement activities
- Adherence to and documentation of equipment cleaning and maintenance schedules as outlined in this manual

- Management of inventory for all study-related consumables
- Adherence to biosafety and infection control principles and practices throughout the study
- Maintenance of patient confidentiality
- Organization of study documentation, and provision of essential documents as applicable to CRAs, auditors, regulatory inspectors, etc.
- Coordination with study-related staff, including routine meetings to review documentation, answer queries, and resolve issues

Supervisory roles may be delegated to qualified personnel as necessary. Documentation of this delegation must be captured in the study documentation.

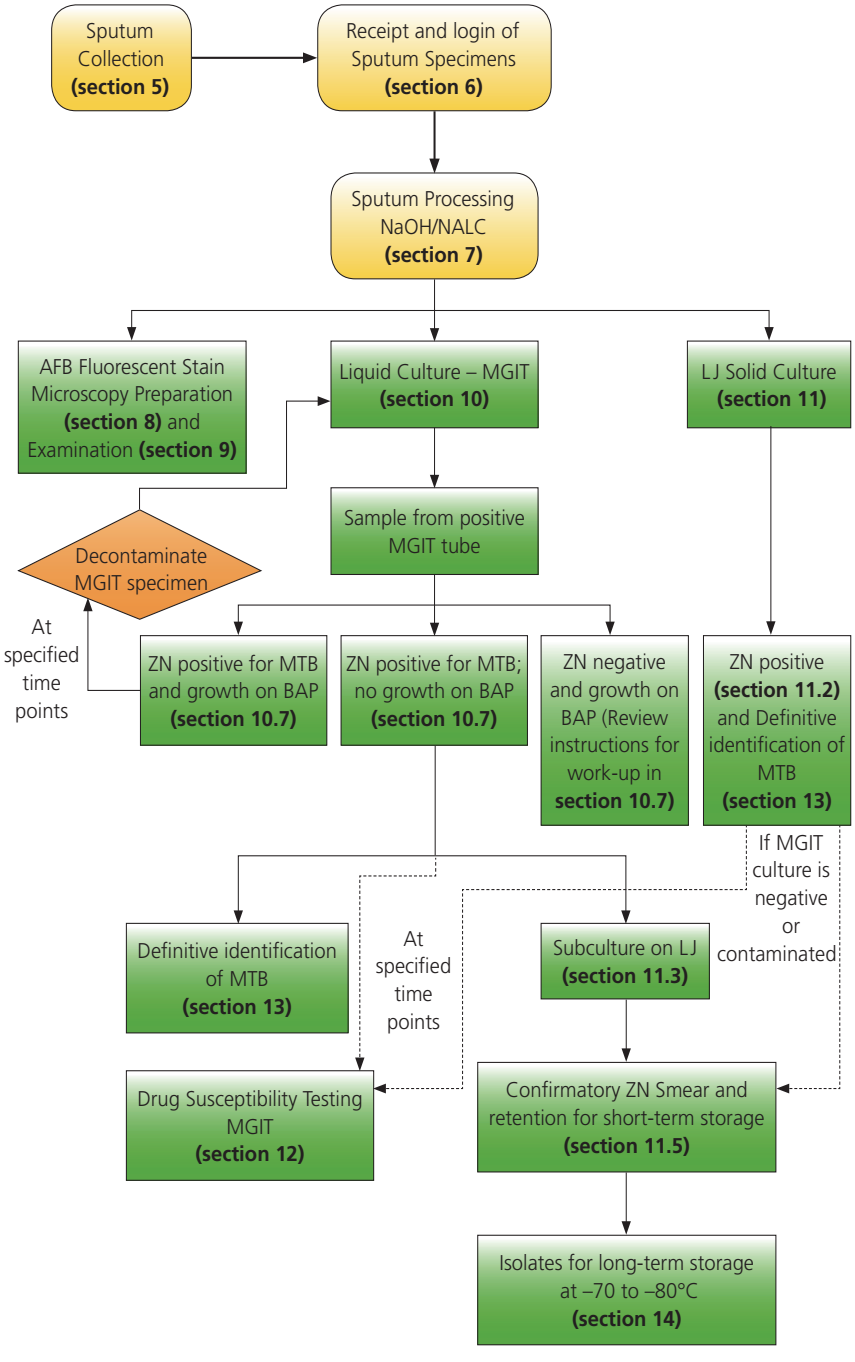
2 SAMPLE SPECIMEN TIMETABLE

Table 2-1 Sample schedule of Microbiological Assessments for All Treatment Groups - Intensive Treatment Period

Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Day/Week																						
Current diagnosis of MDR-TB																						
Sputum AFB smear[a]																						
LJ culture[a]																						
MGIT culture[a]																						
Confirmation of MTB (isolate identification)[b]																						
1st/2nd-line drug susceptibility testing[c]																						
Short/long term storage[d]																						

a **Two** sputum samples will be tested at each scheduled visit except for **Visits 2 and 3** (Day -1 and Day 1; on these days, only one sputum sample will be collected by the site staff). When two specimens are collected, the first specimen will be collected by the patient at home, early in the morning, and preferably prior to the morning meal (except for hospitalized patients - this specimen will be collected by site staff). The second specimen will be collected at the site by the study staff, using induction if the patient is unable to spontaneously expectorate a sample during site collection. If a patient is unable to produce sputum during the visit, two additional attempts to collect the specimen must be made over the next 48 hours.
b Definitive identification of MTB will be performed from both MGIT and LJ media when growth is detected from both.
c Susceptibility testing for first-line anti-TB drugs and key second-line drugs utilized at each site (second-line DST only required for injectable(s) and fluoroquinolone(s)) will be performed with MGIT system.
d **Short-term storage** for MTB isolates recovered from a positive MGIT (or LJ culture if MGIT not available) will be performed by subculturing to LJ slants for storage at room temperature or at 4°C until the end of the study. **Long-term storage** of MTB isolates recovered from positive cultures (MGIT and/or LJ) will be stored in duplicate in 7H9 broth w/ glycerol at –70 to –80°C for six months after the conclusion of the study, unless otherwise instructed by the sponsor.

3 SPECIMEN FLOWCHART



Section 4: Laboratory Biosafety and Infection Control and Section 16: Quality Assurance apply to all procedures in the flowchart.

4 LABORATORY BIOSAFETY AND INFECTION CONTROL

Purpose

Transmission of tuberculosis, including drug-resistant TB, is a recognized occupational risk for laboratory personnel, as well as a hazard to others who may be exposed to infectious aerosols in the laboratory. Tubercle bacilli may be present in sputum, gastric lavage fluids, cerebrospinal fluid, urine, and in a variety of tissues. MTB bacilli may survive in heat-fixed smears and may be aerosolized during the processing of specimens and inoculation of culture media. Although clinical specimens from TB cases contain a low infective dose of MTB, all specimens from suspected or known TB cases must be considered potentially infectious and handled with appropriate precautions. Furthermore, MTB cultures and drug susceptibility testing procedures generate high concentrations of organisms that pose an increased risk of aerosol production. Thus, all aforementioned procedures necessitate special biosafety containment and practices.

Principle

Regardless of the level of risk for spread of TB infection, standards are recommended for all laboratories handling specimens from suspected or known TB cases. These standards are endorsed by WHO, US CDC, and US National Institute of Health (NIH). In addition, Good Clinical Laboratory Practice (GCLP) standards, a set of regulations that guide laboratories conducting clinical trials, require a laboratory design that ensures the safety of personnel and the quality of work. In summary, these standards require:

- Administrative controls, including good lab practices, SOPs, and accident management plans
- Engineering controls, such as a controlled ventilation system
- Use of personal protective apparel/equipment appropriate for the task
- Waste management procedures
- General lab safety procedures – including physical, biohazard, fire, chemical, and electrical safety

Materials/Equipment for all TB Laboratory Procedures

- Class II Biosafety Cabinet (BSC)
- N95, FFP2, or equivalent respirators
- Lab gowns
- Disposable gloves
- Biohazard bags
- Tuberculocidal disinfectant
- Waste receptacles
- Centrifuge with biosafety canisters/lids
- Autoclave
- Autoclave tape
- Spore test for autoclave

4.1 Recommended Facilities and Equipment

- The lab must be contained, i.e., physically separated from other labs.
- Access to the lab must be restricted, preferably through an anteroom.
- Controlled ventilation should be installed which maintains a directional airflow into the lab, supported by a visual monitoring device showing that proper directional airflow is maintained at all times.
- Air from the containment lab should not be re-circulated to other areas in the building – this environment can be achieved through high-efficiency particulate air (HEPA) filtration within the ventilation system.
- Class II biosafety cabinets (BSC; vertical, laminar-flow which blows HEPA-filtered air over work area; UV lamp optional) must be used for all manipulations of clinical specimens and positive cultures.
- BSCs must be certified at least annually by personnel trained in the certification process.
- Clinical centrifuges must be equipped with biosafety canisters (buckets with aerosol-containment lids).
- Microcentrifuges should be operated inside BSC using tubes with caps having O-rings.

4.2 Recommended Biosafety Practices

- Take caution when performing aerosol-generating procedures such as centrifugation, vortexing, mixing, pipetting, pouring, and inoculation of media. For example:
  - Delay opening caps until aerosols have settled
  - Open centrifuge canisters only inside the BSC
  - Use pipettes that are easy to control
  - Eject micropipette tips down inside discard bucket
- Heat-fix slides on a warmer in the BSC, heating them at 65-75° C for at least two hours before handling outside the BSC. Heat-fixed smears may contain viable tubercle bacilli, but they are not easily aerosolized if dried on a slide warmer for two hours.
- Inside the BSC, keep arms parallel to the work surface, work in the center, and minimize arm movements; once beginning work, do not move hands out of the hood until work is completed. These precautions will minimize interruption of airflow inside the BSC.
- Keep the amount of equipment inside the BSC to a minimum so as to not interfere with the airflow pattern.
- Disinfect the BSC and all work surfaces with a tuberculocidal disinfectant (capable of killing MTB), before and after every procedure.
  - The most common tuberculocidal disinfectants are bleach (hypochlorite) and phenol-based disinfectants. Diluted (working) bleach solutions must be prepared daily and stay at or above 0.5 % chlorine – undiluted commercial bleach is usually 4-5 %. Similarly, phenol-based disinfectants must be diluted daily, preferably with deionized (not "hard") water, to 2-5 %. Both types of disinfectants are only effective if left in contact with the contaminated material for at least 15 minutes. Check the manufacturer's recommendations for the specific disinfectant to be used.
- Place all wastes containing MTB in a leak-proof container or autoclavable plastic bag that contains disinfectant solution which can be sealed before being removed from the BSC and autoclaved.

- The autoclave should be monitored with a spore test at least monthly to ensure that sterility is achieved.
- Avoid practices that can result in spills, e.g., hand-carrying tubes, vials, and bottles, or improperly stacking racks or baskets. All tubes, plates, and other containers should be transported on carts in protected racks or baskets.
- Write a procedure for the appropriate handling of a spill, both inside and outside the BSC. The procedure should include:
  - Inside the BSC: the BSC should continue to run and the appropriate disinfectant should be applied to the spill for at least 20 minutes, after which the items can be transported to the autoclave. If a liquid culture is spilled, all personnel should exit the containment room, and allow the BSC to run for at least 30 minutes before entering the room.
  - Outside the BSC (but within the lab): all staff should leave the room for at least 30 minutes to allow the aerosols to settle. The person cleaning the spill should wear a respirator when disinfecting the area. If the lab is a biosafety level 3 (BSL3) laboratory, all staff should leave the room, and the person disinfecting the spill can enter immediately (wearing a respirator), without waiting for the aerosols to settle.

4.3 Recommended Personal Protective Equipment

- Staff working in the containment lab must wear protective laboratory clothing such as a solid-front or wrap-around gown. If scrub suits are worn, protective gowns should be worn on top. The scrub suits should be changed daily. The protective gown must have long sleeves with snug (knit) cuffs.
- Gloves must be worn and must be long enough to overlap the sleeves of the gown.
- Hair covers (caps) and shoe covers are recommended.
- All outer protective clothing must be removed when leaving the containment laboratory.
- Respiratory protective devices are highly recommended while working in the containment lab. Any respirator conforming to the National Institute for Occupational Safety and Health (NIOSH) N-95 rating, European Committee for Standardization 'FFP2' rating, or equivalent is acceptable.
- Respirator protection is more than just wearing a mask! Attention must be given to:
  - Selecting the appropriate respirator for the individual
  - Conducting fit-testing
  - Training personnel on the use, fit checking, and storage of the respirator

NOTE: Laboratory infections are nearly always caused by poorly monitored BSCs or a BSC in which normal aerosol containment capability is compromised, thereby permitting escape of droplet nuclei. A respirator can serve as an additional barrier to reduce the likelihood that tubercle bacilli will enter the lung.

4.4 Personnel Protection - Training and Monitoring

- All personnel working in the containment lab must have proper training on biosafety procedures, use of personal protective equipment, and how to monitor all equipment (especially the BSC) for proper operation. Documentation of this training must be kept with personnel training records.
- Staff should receive frequent re-training and be monitored to ensure compliance.
- Staff should be participating in a TB screening program, per the laboratory's national guidelines for preventing TB infection.

5 SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Purpose

Proper collection and transport of sputum specimens is required to ensure quality laboratory results. Adherence to procedural details will result in collecting adequate and quality sputum specimens for analysis in the mycobacteriology laboratory and maintaining correct identity of the specimen. **The procedure below details sputum collection at the site by site staff.** Patient sputum collection instructions are documented elsewhere.

Principle

Sputum specimens will be collected according to the visit schedules in Section 2: Sample Specimen Timetable. On visits 2 and 3, only one sputum specimen will be collected from the patient. Thereafter, two sputum specimens will be collected at each timepoint. The first specimen, considered to be the 'morning' specimen (collected prior to the morning meal and any dosing), will be labeled "**Sputum Specimen #1**". The second specimen, considered a 'spot' specimen, will be labeled "**Sputum Specimen #2**".

Sputum Specimen #1

This specimen will be collected by the patient at home, *unless s/he is hospitalized*. The patient will use a special sputum collection container that minimizes contamination and leakage and eliminates the need to transfer sputum from a container to a 50 ml tube. The container has the following parts:

- 50 ml centrifuge tube and cap
- Built-in funnel
- Outer container and lined cap
- Sealed label

If the specimen is collected by the site, a sterile 50 ml centrifuge tube will be used. In either case, the specimen will be sent by the site to the laboratory.

Sputum Specimen #2

This specimen will be collected by the site staff in a sterile 50 ml centrifuge tube.

PROCEDURE

Materials

- Sterile, disposable, single-use, screw-capped, conical centrifuge tubes (50 ml)
- Sponsor-provided Sputum Collection Kit
- Permanent marker
- Study Labels
- Disposable gloves
- Refrigerator with detached thermometer
- Cooler
- Cold packs

Forms

- Laboratory Specimen Requisition Form – this is a site-specific form used for routine laboratory requests.
- Appendix A - Specimen Transfer Form
- Appendix D - Equipment Temperature Record Form (or use site-specific form, if available and equivalent)

5.1 Sputum Collection

1. Collect specimens in appropriate sterile, disposable containers. Screw caps must fit tightly to avoid leakage. 50 ml polypropylene centrifuge tubes are preferred.
2. Discuss the following collection procedures with the patient:
   - Emphasize the nature of the desired specimen.
     – Inform the patient that nasal secretions and saliva are not sputum.
     – Explain that the desired specimen is produced by a deep cough and is thick, mucoid, white-yellow, and sometimes blood-tinged. It is from the lower airways and lung.
   - Instruct the patient not to touch the inside of the collection container or lid with their fingers or other objects.
3. Positively identify the patient. Prepare two study labels (three for patient-collected samples) with the screening and/or subject ID number, date and time of collection, sputum specimen number (#1 or #2, or N/A if Visit 2 or Visit 3) and visit number for which the specimen is being collected. Place one study label on tube/container in which the sputum will be collected.
4. If able, instruct the patient to stand.
5. Give the patient a glass of water (bottled or boiled) to rinse the mouth free of food particles. Instruct the patient to rinse twice.
6. Instruct the patient to produce sputum, after rinsing mouth as described above. Use a demonstrator glass and tube/container to show the patient the procedure if this will help.
   - Take a deep breath.
   - Hold breath for a moment.
   - Cough deeply and vigorously at the same time the breath is coming out.
   - Release sputum into the labeled tube/container by holding it to the lower lip and gently releasing the specimen.
   - Close the tube/container tightly with the screw-on lid without touching the inside of the lid. Avoid spills or soiling the outside of the container.
7. If the patient cannot cough spontaneously, instruct the patient to take several deep breaths and hold the breath momentarily. Repeating this several times may induce coughing.
8. If a patient is unable to spontaneously expectorate a sputum sample, an attempt will be made to induce sputum production by aerosol inhalation. Contact the study physician to conduct this procedure. Sputum induction will be performed according to the standard procedure at the site. The Laboratory Specimen Requisition Form and the Appendix A: Specimen Transfer Form must also accompany the appropriately-labeled induced sputum to the lab. Clearly mark that the sputum has been induced under the section labeled "Sputum Induced?" by circling 'Yes' or 'No'.

9. After the specimen is collected, complete the following:

    - Laboratory Specimen Requisition Form – all relevant fields must be completed.
    - Appendix A– must contain the following:
        – site name and number
        – screening ID number and/or subject ID number, subject initials
        – visit number
        – date and time of collection
        – specimen number (#1, #2, or N/A for V2 or V3)
        – whether sputum was induced or not
        – who the specimen was collected by (either patient or site staff)
        – total volume
        – time sputum was at room temperature
        – study label
        – date/time of dispatch to laboratory
        – mode of transport
        – name of person collecting (or receiving if patient-collected sample) the specimen
        – name and signature of person completing the form
        – transport/courier information (to be completed by driver, or site personnel can complete this section if a courier is used)

10. The laboratory section will be completed by laboratory staff when they receive the specimen.

5.2 Storage of Sputum Specimens

1. Check that the tube is tightly capped, properly labeled, and that the screening and/or subject ID on the tube matches the screening and/or subject ID on the requisition and Appendix A forms.

2. Refrigerate specimens at 2-8°C until ready for transport to the laboratory. Refrigeration reduces the growth of contaminants in the specimen. If a refrigerator is not available, specimens can be held in coolers with ice packs.

NOTE: Refrigerators in which sputum specimens are stored must be monitored with daily temperature readings using an internal detached thermometer. The acceptable range is 2-8°C. Record the temperature daily on the appropriate Equipment Temperature Record Form (Appendix D).

5.3 Transport of Sputum Specimens

1. Specimens should be delivered to the laboratory the same day as collected, if the study site is in the same city as the mycobacteriology laboratory.

    - Sputum specimens collected in the morning should reach the laboratory in the morning or early afternoon so that they can be processed the same day.
    - Sputum specimens collected in the late afternoon should either arrive to the laboratory on the same day, for processing the next day, or early the following morning, for processing the same day.
    - Sputum specimens collected at the very end of the day when transport is not available may be stored in the site's refrigerator overnight, but must be delivered to the laboratory early the following morning.

2. If specimens are transported longer distances, they must be delivered as soon as possible, but no later than 48 hours from time of collection. These specimens must be stored in the refrigerator and transported on ice. Arrange a pick-up time which will allow the specimens to be delivered on a week day, unless the laboratory is open on Saturdays and delivery can be made on Saturdays. Notify lab if a Saturday delivery is planned.

3. Before transporting from the clinic, a designated staff member of the study team must verify for each transport box that:

    - The total number of sputum tubes/containers in the box corresponds to the number of Laboratory Specimen Requisition and Appendix A forms (one of each form per specimen).
    - The screening ID number or subject ID number on the container/tube corresponds to that on the Laboratory Specimen Requisition and Appendix A forms.
    - The Laboratory Specimen Requisition and Appendix A forms contain all the requested information for each patient.
    - The "Dispatch Section" of Appendix A must be completed and signed by the individual who collected the specimen or the person responsible for handing off the specimens to the transport service.

4. When this verification is complete, put the specimens in the transport container with ice packs. For transport on site or within the city, each specimen should be in a sealed plastic bag. If bags are not available, check to ensure that caps are tightly closed and place containers or tubes in a rack to keep upright during transport. For longer distances, each specimen must be packaged in a separate sealed plastic bag before placing in a transport cooler with ice/cold packs.

5. Place the Laboratory Specimen Requisition and Appendix A forms in an envelope and attach to the outside of the transport container. If the courier service requests that the forms be enclosed, then place envelope in a sealed, leak-proof bag and place the bag inside the transport container.

6. The name and signature of the transport driver, date and time of pick-up are recorded in the "Transport Section" of Appendix A. If a courier service is being used, this section can be filled in by the site study team member, and the name of the courier and tracking number provided, instead of the driver name and signature.

6 RECEIPT AND LOGIN OF SPUTUM SPECIMENS IN THE LABORATORY

Forms

- Laboratory Specimen Requisition Form – this is a site-specific form used for routine laboratory requests
- Laboratory Specimen Logbook/Registry– this is a site-specific form used for routine laboratory requests. If possible, use a separate logbook for study patients and non-study patients.
- Study Labels
- Appendix A - Specimen Transfer Form
- Appendix B - Study Source Document Worksheet

6.1 Receipt of Sputum Specimens

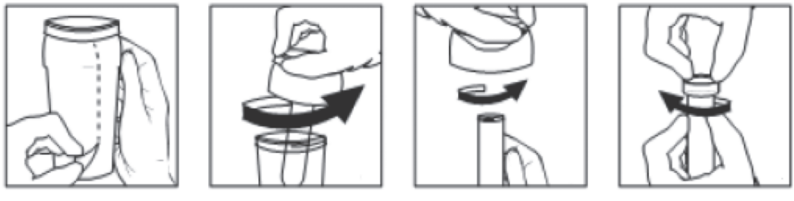
6.1.1 Sputum Specimen #1 – patient-collected sputum

1. Patient-collected specimens will arrive in a sealed, plastic biohazard bag. Study labels will be placed on the cap of the outer collection container and on the side of the plastic label. Upon arrival at the laboratory, specimen labels must be verified with the corresponding Laboratory Specimen Requisition Form and Appendix A.
    - Check the plastic bag for any leaks or cracks in the outer container. If a leak is seen, do not open the plastic bag. Autoclave the container and bag and discard. Record this occurrence in the laboratory "Comments" section on Appendix A and in the Lab Specimen Logbook/Registry. It is important to notify the site about the reason for any leaks. If a container is cracked or not closed properly, site staff can re-educate patients and/or the courier.
    - Open the plastic biohazard bag, remove the sputum container, and verify the specimen information on the study label against the Laboratory Specimen Requisition Form and Appendix A. **Do not remove the white cap or the plastic label from the sputum collection container unless specimen receipt is performed in a biosafety cabinet.**
    - The individual receiving the specimens records their name, signature and date/time of receipt, as well as a description of the specimens, on the "Laboratory Section" of Appendix A.

2. If the laboratory finds a mislabeled or mismatched specimen and requisition, the following procedures must be followed:

- For a specimen with an ID on the tube and a mismatched, incomplete, or missing requisition:
  - The laboratory will contact the site to obtain any needed information before the specimen is processed.
  - Specimens without requisitions are unacceptable, and will not be processed until a requisition form is received. The laboratory will contact the site.
- For unlabeled specimens: **(There are no exceptions to this policy.)**
  - The laboratory will not process unlabeled specimens, and specimens must always be labeled at the time of collection.
  - Notify the site when an unlabeled specimen is received.
  - The specimen will remain in the laboratory, and must be labeled by someone from the site who will sign a statement taking responsibility for the labeling. This statement must be filed with the Appendix A form in the study laboratory binder.
  - The fact that the specimen was received unlabeled and subsequently labeled will be entered in the laboratory "Comments" section of the Appendix A form, and in the lab specimen logbook or database (if available).

3. Collection containers must be opened in a biosafety cabinet. Only open one specimen container at a time to prevent mislabeling.



- Remove the plastic label from the container by tearing along the perforations.
- Unscrew the funnel portion from the container.
- Remove the 50 ml centrifuge tube and close tightly using the cap provided.
- Immediately prepare a study label with the information in **Section 6.2**: Login of Sputum Specimens and place on the 50 ml tube.
- Container, funnel, and white cap must be autoclaved prior to disposal.

4. Sputum specimens must be 2-3 ml in volume. If the specimen is less than 2ml, discuss the volume with the lab director and determine if the quality and volume are sufficient for processing.

5. Estimate the volume of each specimen by comparing with a 50 ml tube that has been calibrated with known volumes of water. This volume must be checked against the volume recorded on Appendix A and adjusted, if necessary, by drawing a single line through the written volume, dating, and initialing. Record the new volume in the appropriate location on the form.

6. Evaluate the quality of each sputum specimen and record on Appendix B. Those with noticeably high salivary content will be noted but processed (a watery specimen is an indication of high saliva content).

7. Compare the date/time of collection on Appendix A with the current date/time. Sputum specimens must be processed within **72 hours** of collection.

6.1.2 Sputum Specimen #2 - site-collected sputum (these instructions also apply to site-collected Sputum Specimen #1)

1. Site-collected sputum specimens will arrive in a 50 ml tube. Upon arrival at the laboratory, verify specimen labels with the corresponding Laboratory Specimen Requisition Form and Appendix A.

    - Check for any leaks or cracks in the tube. If a leak is seen, decontaminate the container in an autoclave and discard. Contact the site immediately and request that another sputum specimen be collected. Record this occurrence in the laboratory "Comments" section on Appendix A and in the Lab Specimen Logbook/Registry.

    - The individual receiving the specimens records their name, signature, and date/time of receipt, as well as a description of the specimen, on the "Laboratory Section" of Appendix A.

2. Follow steps 2, 4, 5, 6, and 7 above.

    Note: Induced sputum may appear watery and resemble saliva due to the method of collection. These specimens are acceptable for testing. Specimens that are visibly contaminated with food particles should only be processed if the patient is not accessible to provide another specimen within 48 hours of the scheduled visit. Call the site immediately to instruct the patient to try again with a new pre-labeled specimen container. Record this information in the "Comments" section on the Appendix A form.

6.2 Login of Sputum Specimens

1. Specimens, Laboratory Specimen Requisition Forms, and Appendix A forms are transferred to the mycobacteriology lab if received in a separate receiving area. Specimens must be stored at 2-8°C if not processed immediately.
2. Assign a unique laboratory accession number to each specimen (note that sputum specimen #1 and sputum specimen #2 must have separate accession numbers). This may take place in the general lab receiving area or in the mycobacteriology lab.
3. Record patient and specimen information in the lab database or specimen log book according to the laboratory's standard operating procedure. Note the study visit number for which the specimen has been collected to ensure the appropriate work-up of a positive culture.
4. Place laboratory accession number on the study specimen label and on the Laboratory Specimen Requisition, Appendix A, and Appendix B forms.
5. The study-specific labels (provided by the Sponsor) will be used to label tubes for all subsequent downstream processing of this specimen - microscope slides, LJ tubes, MGIT tubes, BAP plates, cryotubes, etc. - and will be affixed to the Appendix B form. **These labels must be completed with the following information: lab accession number, screening ID number and/or subject ID number, visit number, sputum specimen number (#1 or #2, unless specimen is from V2 or V3) site number, and applicable date (e.g., the collection date is written on the Appendix B label, the date of freezer storage on the cryotube label, etc.).**

    Screen ID: _____ Subj ID: _____

    V#: _____ Specimen#: _____ Site#: _____

    Date: _____ Lab access #: _____

6. Original Appendix A and Appendix B forms will be stored at the laboratory.
7. Send **completed** copies of Appendix A (signed by the laboratory technician) and Appendix B (signed by the lab supervisor) to the study coordinator to be stored with the trial documents.

8. In addition, send a copy of Appendix B to the study coordinator whenever new information is added. These routine transmittals allow the study coordinator to update the study database with new microbiology lab information. The supervisor or technician must initial and date in the margin next to the new information being sent so updated information is readily evident.

NOTES:
- All specimens for smear and culture are expected to be processed on the day of receipt in the laboratory, especially if on the following day(s) the lab is closed.
- Specimens must be refrigerated if not processed within one hour.

6.3 Study Data Reporting

Record the following on Appendix B:

- lab accession number
- site number
- screening ID number and/or subject ID number, patient initials
- visit number
- sputum specimen number (#1, #2, or N/A)
- date and time specimen was received, specimen volume, and type of specimen (i.e. induced or not, patient-collected or site-collected)
- name of person collecting the specimen
- any comments

7 PROCESSING SPUTUM FOR SMEAR MICROSCOPY AND QUALITATIVE CULTURE

Purpose

Processing sputum specimens has two objectives: decontamination of bacteria other than mycobacteria and liquefaction of mucous and organic debris in the specimen. Although there are several techniques available, none of them are ideal, meaning none of them will selectively destroy only contaminating flora and achieve complete liquefaction of the specimen. A reasonable compromise is to destroy as much of the contaminating bacteria as possible while harming as few mycobacteria as possible. All sputum specimens will be processed in this manner for preparation of AFB smears and liquid (MGIT) / solid (LJ) cultures.

Principle

N-acetyl-L-cysteine (NALC), a mucolytic agent, is used for rapid digestion, which enables the decontaminating agent, NaOH, to be used at lower final concentration (in sputum). NALC loses activity rapidly in solution, so it is made fresh daily. Sodium citrate exerts a stabilizing effect on the NALC by chelating heavy metal ions present in the specimen. The phosphate buffer neutralizes the NaOH and dilutes the homogenate to lessen the viscosity and specific gravity prior to centrifugation. Mycobacteria have a low specific gravity and may remain buoyant during centrifugation. A relative centrifugal force of 3,000 *xg* (**not** 3,000 rpm – the centrifuge must be calibrated) for 15 minutes is adequate to sediment mycobacteria. The rate at which mycobacteria sediment is critically dependent on time of centrifugation and relative centrifugal force applied to the specimen. A longer centrifugation time can offset a lower relative centrifugal force, but increased centrifugation time increases the temperature of the specimen, which leads to additional killing of mycobacteria (hence, a refrigerated centrifuge is highly recommended).

Procedure

Materials

- Tuberculocidal disinfectant
- 70% ethanol
- Waste receptacle, splash-proof for liquids
- Discard bucket with biohazard bag insert, containing appropriate disinfectant
- Sterile cylinder and break-resistant glass bottle to prepare NaOH/NALC-Na citrate
- Clean cylinder, flask, and beaker to prepare phosphate buffer
- 50 ml conical polypropylene tubes with plastic screw cap
- 60 ml Nalgene tubes with plastic screw cap, autoclavable
- NALC powder
- 6% NaOH
- 2.9% Na citrate
- Phosphate buffer pH 6.8 (or components if made in-house)

- Analytical balance
- Precision balance
- Weigh boats
- Vortex mixer
- Clean rack for 50 ml centrifuge tubes
- Refrigerated centrifuge with covered bucket inserts for 50 ml conical tubes
- Timer
- 2 ml disposable, serological pipette, sterile, individually wrapped
- Pipette aid (Drummond Scientific, Portable Pipet-Aid XP or similar)
- Sterile, transfer pipettes with graduations marking volume (individually wrapped)
- Magnetic stirring bar and stirrer
- pH meter or appropriately sensitive pH paper
- Slide warmer (hot plate)
- Study labels
- Pencil for labeling slides
- Permanent marker
- Shaker
- Microscope slides, frosted one end, new and clean
- MGIT tubes
- LJ tubes
- Paper towels

Forms

- Laboratory Specimen Requisition Form– this is a site-specific form used for routine laboratory requests.
- Specimen Processing Worksheet/Workbook – this is a site-specific form used for routine laboratory tests.
- Appendix D - Equipment Temperature Record (or use site-specific form, if available and equivalent)
- Appendix B - Study Source Document Worksheet

7.1 Preparation of NaOH/NALC-Na Citrate Solution and Phosphate Buffer

Advance Preparation of Solutions:

6% Sodium Hydroxide, 2.9% Sodium Citrate

1. To prepare 1 L digestant-decontaminant solution:
   - 6% NaOH: dissolve 30 g NaOH in 500 ml distilled water.
   - 2.9% Na citrate: dissolve 14.5 g Na citrate dihydrate in 500 ml distilled water.

- Sterilize each reagent by autoclaving at 121°C for 15 minutes.
- Label containers with the buffer name, date prepared, expiry date, batch lot number, and technician initials.
- Store reagents refrigerated until needed.

2. Alternatively, the solutions may be mixed, sterilized, and stored in screw-cap containers for later use. For example:
    - In a 4 L flask combine 1 L of 6% NaOH and 1 L of 2.9% sodium citrate.
    - Mix well and aliquot into 500 ml bottles.
    - Sterilize by autoclaving at 121°C for 15 minutes.
    - Label containers with the buffer name, date prepared, expiry date, batch lot number, and technician initials.
    - Store reagents refrigerated until needed.

Phosphate Buffer Solution

1. To prepare 1.5 L of phosphate buffer:
    - Combine 7.1 g disodium phosphate (Na_2HPO_4), 6.8 g monopotassium phosphate (KH_2PO_4), and 1,500 ml distilled water in a 2 or 4 L flask.
    - Stir with magnetic stirring bar on magnetic stirrer.
    - Check pH, which should be 6.8. Adjust if necessary. Add disodium phosphate to raise pH; add monopotassium phosphate to lower pH.
    - Using a smaller flask or beaker, dispense buffer into smaller volume containers for storage (ideally, 50 ml are dispensed into 60 ml sterile Nalgene tubes; alternatively, 100 - 200 ml sterile containers can be used).
    - Label containers with the buffer name, date prepared, expiry date, batch lot number, and technician initials.
    - Cap the tubes and sterilize in the autoclave. These smaller aliquots will be used to wash the digested-decontaminated specimen and resuspend the pellet.
2. Alternatively, stock solutions of disodium phosphate and monopotassium phosphate can be made in large volumes, sterilized, and stored separately. When ready to use, mix equal volumes in a sterile container and check pH using aseptic technique. Then proceed to dispense 50 ml into 60 ml sterile Nalgene tubes, as described above.

<u>Daily Preparation of Solutions:</u>

NaOH/NALC-Na citrate

1. To prepare NALC-NaOH working solution:
    - Just before use, determine how much reagent will be needed for the day's work.
    - If solutions are stored separately, mix equal volumes of the NaOH and Na citrate solutions in a sterile flask using a sterile cylinder, e.g., 100 ml of each. The resulting solution contains 3% NaOH.
    - Using the table, add the appropriate amount of 0.5% NALC to the NaOH-Na Citrate solution. For example, if a total of 200 ml NaOH-Na Citrate solution is needed, add 1 g NALC (see table below), to the flask. Mix gently – do not shake vigorously.

- Store NaOH/NALC solution at 2-8°C between processing batches, as NALC loses mucolytic activity on standing at room temperature. However, ensure the solution is brought to room temperature prior to using on subsequent specimens.
- If more or less of this solution will be used in one day, prepare the appropriate volume needed. Discard any unused NaOH/NALC solution at the end of the day.

2. Pour a smaller volume of this working solution into a 50 ml conical tube and use this smaller aliquot for digesting-decontaminating specimens. **This technique reduces the potential for contaminating the larger stock bottle.**

Table 7.1 Preparation of NaOH/NALC-Na citrate digestant solution

Volume of digestant needed (ml)	6% NaOH	2.9% Na citrate	Amount NALC to add (grams)
50	25	25	0.25
100	50	50	0.50
200	100	100	1.00
250	125	125	1.25
500	250	250	2.50
1000	500	500	5.00

7.2 Preparation of BSC and Special Microbiology Practices

All processing must be done in a biological safety cabinet (BSC) using full PPE.

1. Before processing the day's specimens, clean BSC surfaces with 70% ethanol and let stand 3 minutes prior to drying with a paper towel.
2. Prepare a splash-proof waste receptacle with tuberculocidal disinfectant at the appropriate concentration and place inside BSC for disposal of liquids.
3. Place paper towels soaked in tuberculocidal disinfectant over the entire work surface inside the BSC.
4. Place a discard bucket with a biohazard bag containing tuberculocidal disinfectant inside BSC for disposal of contaminated materials.
5. **Do not work with more specimens than can be placed in the centrifuge at one time. When processing multiple batches on the same day, clean the BSC with disinfectant, turn on UV lights (if available) for 20 minutes, and allow air to circulate in the BSC for 20 minutes between batches, prior to repeating steps 1-4.**
6. Place specimen tubes at least one space apart in rack to aid in preventing contamination.
7. Work methodically with the tubes on one side and discard buckets close to the specimens, to avoid spillage/confusion of samples.
8. To reduce the risk of cross-contamination, ensure that reagent containers do not come in contact with the edge of the specimen container.
9. Remove only one cap at a time from the tubes (50 ml specimen tube, culture tubes) to avoid cross-contamination and misplacing the caps on the wrong tubes.
10. Ensure that tubes, bottles, racks, pipette aid, etc. that are removed from the safety cabinet are free from any droplets/potential contaminants. If necessary, wipe the rack or tube with a paper towel soaked in tuberculocidal disinfectant prior to removal from the BSC. In addition, if any suspicion of droplets from a specimen is seen on gloves, wipe gloves with a disinfectant-soaked towel and change gloves prior to processing additional specimens.
11. Upon completion of work, place paper towels in discard bucket. Wipe all BSC surfaces with 70% ethanol, let stand 3 minutes and wipe dry. If BSC is turned off in the evening, be sure to leave on for 1 hour before turning off. If available, turn on UV light inside BSC for at least 1 hour.

7.3 Specimen Digestion-Decontamination

1. Follow the login procedure for specimen registration described in **Section 6**: Receipt and Login of Sputum Specimens in the Laboratory.
2. Record all specimens processed in a batch using the Lab Processing Worksheet/Workbook. Also, record the technician/technologist processing each batch.
3. Label slides and tubes, using the study-specific labels provided, with information described in **Section 6.2**: Login of Sputum Specimens.
4. Allow refrigerated specimens and reagents to come to room temperature before testing.
5. The ideal maximum volume of a sputum specimen is 10ml. If the volume is greater than 10ml, vortex the specimen and remove the excess volume with a pipette to bring the total volume to 10 ml prior to processing.
6. Using a transfer pipette, add to the sputum tube a volume of NaOH/NALC-Na citrate solution (prepared above) that is equal to the specimen volume (1:1 volume) to the centrifuge tube. Use a new sterile pipette for each specimen (**The final NaOH concentration at this stage is 1.5%**).
7. Close tube tightly and vortex the suspension until liquefied (15-30 seconds). Invert the tube several times so the tube walls and cap are exposed to the NaOH/NALC-Na citrate solution.
8. Start the timer for 15 minutes when the digestion-decontamination solution is added to the first specimen. Place tube in rack on shaker platform to improve homogenization:
    - Adjust speed of rotator so that it mixes the specimen well while ensuring the entire sample is exposed to the digestion-decontamination solution.
        - Start with an rpm of 60, increasing speed in small increments, e.g., 5 rpm, until a gentle, but even, mixing of the sample occurs, usually between 60 and 80 rpm depending upon the instrument.
        - **Do not agitate the tube too vigorously;** extensive aeration causes oxidation of NALC, making it ineffective for liquefying sputum.
        - Once the ideal speed is identified, use for processing of all sputum samples.
    - If a shaker is unavailable, vortex the tubes gently (to mimic the shaking action) 2-3 more times during the incubation period.
9. Repeat steps 6-8 for subsequent specimens at 30 sec or 1 minute intervals.
10. Make sure the specimen is completely liquefied. If still mucoid after 10 minutes, add ~ 50 mg of NALC powder and vortex 15-30 sec.
11. When time has elapsed, remove tube from shaker. Add phosphate buffer (pH 6.8) to the 50 ml mark on the centrifuge tube using pre-dispensed, 50 ml aliquots of buffer. Mix by inversion or vortex. Discard unused buffer.
12. Continue to add phosphate buffer to all specimens at 30 sec or 1 minute intervals (as above), so that each specimen is ONLY exposed to digestion-decontamination solution for a maximum of 20 minutes. **It is very important that the phosphate buffer is added within 20 minutes of adding the digestion-decontamination solution**, since mycobacteria can be killed off if exposed to NaOH longer than 20 minutes.
13. Transfer tubes in a 50 ml tube rack to the centrifuge.
14. Place tubes in centrifuge buckets, ensuring they are equally balanced and the biosafety covers (lids) are properly placed on each of the buckets.

15. Centrifuge at 3,000 *xg* (note this is **not** 3,000 rpm) for 15 min at 4-12°C. The 15 minute centrifugation period should begin only **after** 3000 *xg* speed has been achieved.

16. Once centrifugation is complete, remove the centrifuge buckets without delay and carry to the BSC prior to opening the biosafety covers.

17. Carefully decant the supernatant into a splash-proof container containing a tuberculocidal disinfectant, taking care not to disturb the sediment at the bottom of the tube.

18. Add sterile phosphate buffer (pH 6.8) (from individual 50 ml aliquots) to the pellet to a final volume of **2.5 ml**, and resuspend the sediment using a pipette -- gently aspirating and expelling buffer. If necessary, compare the volume in the tube to a reference 50ml tube with a pre-measured 2.5 ml volume of water. Use a new pipette for each specimen.

19. Use the suspended pellet for culture in the MGIT BACTEC 960 TB System **Section 10**: Liquid Culture – Mycobacteria Growth Indicator Tube (MGIT), and culture on LJ media **Section 11**: Solid Culture: Lowenstein Jensen (LJ) Media. The same pipette can then be used to prepare the smear for AFB microscopy **Section 8**: AFB Microscopy Preparation and Staining. Inoculate culture media and prepare smear for AFB stain immediately after suspending the pellet, using the same pipette.

20. When finished, dispose of the pipette into the biohazard discard bucket.

21. It is recommended to store any remaining sediment at 2-8°C, at least until the sputum smear is confirmed as acceptable. Autoclave sputum sediment before discarding.

7.4 Internal Quality Control and Data Monitors

1. Record preparation information for each new batch of digestion/decontamination reagents, such as lot numbers, expiry dates, pH of buffer, etc. See **Section** 16.3 for more details.

2. Include one negative control sample (to control for cross-contamination), and one positive control sample (to verify accuracy of methods) weekly with a batch of specimens. These samples must be included near the end of the batch and handled as patient specimens being processed for smear microscopy and culture (MGIT and LJ media). See **Section** 16.4.3.1 for more details.

7.5 Study Data Reporting

Record the date and time of sputum processing, as well as the character (quality) of the sputum, on Appendix B.

8 ACID-FAST BACILLI MICROSCOPY (AFB) PREPARATION AND STAINING

Purpose

The purpose of AFB microscopy is to detect acid-fast bacilli (AFB) by microscopic examination of clinical specimens and cultures. Both living and dead (viable and non-viable) bacilli will stain and be counted. A semi-quantitative grading system is used to report the number of AFB observed in stained smears.

All sputum smears are prepared from decontaminated and concentrated specimens (**Section** 7: Processing Sputum for Smear Microscopy and Qualitative Culture). These smears are stained with fluorescent stains, either auramine O or auramine/rhodamine. The Ziehl-Neelsen stain can be used to confirm fluorescent smear results, but these results will not be reported. The Ziehl-Neelsen stain is used to confirm the presence of AFB in positive cultures (MGIT, LJ).

If an alternative fluorescent stain such as acridine orange is used, microscopic examination of the sputum smear must conform to the reading and reporting procedures described in **Section** 9: Acid-fast Bacilli Microscopy (AFB) Examination.

Principle

For the fluorochrome stain, the principle of stain, decolorizer and counterstain is the same as for Ziehl-Neelsen staining. With auramine O stain, organisms fluoresce bright yellow, non-specific debris stains pale yellow, and the background is almost black. With auramine/rhodamine stain, organisms fluoresce yellow-red in an almost black background. Fluorochrome stain is more sensitive than Ziehl-Neelsen because the smear can be examined under a lower power, thus more fields can be read in the same amount of time, and the bacilli stand out brightly. The Ziehl-Neelsen method uses a carbol fuchsin stain, acid alcohol decolorizer, and methylene blue counterstain. Acid-fast organisms stain red, while the background of debris stains blue. The ZN stain confirms the acid-fast property of mycobacteria.

Procedure

Materials

- Tuberculocidal disinfectant
- Waste receptacles (including splash proof receptacle for liquids)
- Discard bucket with biohazard bag insert, containing appropriate disinfectant
- Paper towel soaked in appropriate disinfectant
- Microscope slides, frosted at one end, new and clean
- Pencil for labeling slides
- Study labels
- Hot plate or slide warmer
- Bunsen burner (or spirit lamp)
- Sterile, transfer pipettes with graduations marking volume (individually wrapped)
- Sterile loop or disposable applicator stick

- Ziehl-Neelsen stain (carbol fucshin, 3% acid alcohol, methylene blue)
- Auramine stain (auramine O, 0.5-1% acid alcohol, 0.5% potassium permanganate) **or**
- Auramine/Rhodamine (auramine/rhodamine, 0.5-1% acid alcohol, 0.5% potassium permanganate) **or**
- Acridine orange stain (acridine orange solution in barbitone buffer, 2N H_2SO_4, acid alcohol decolorizer)
- Staining sink
- Staining rack
- Slide drying rack
- Forceps
- Timer
- Vortex mixer
- Distilled water
- Wash bottle

Forms

- Appendix E - Reagent/Media QC Form
- Appendix C - Daily AFB Staining QC Form

8.1 Preparation of Smears from Processed Sputum

The slides must remain in the biological safety cabinet until they have dried.

1. Label the frosted end of the slide in pencil with the laboratory accession number, screening ID number and/or subject ID number, visit number, sputum specimen number (#1 or #2, unless specimen is from V2 or V3), and date.
2. Working in a biological safety cabinet, vortex the decontaminated sediment (see **Section** 7: Processing Sputum for Smear Microscopy and Qualitative Culture) to mix thoroughly.
3. Use a transfer pipette to place ~100 µl (2 drops) of well-mixed resuspended pellet from the digested-decontaminated specimen onto the slide, spreading over an area approximately 1 x 2 cm. Air-dry the smear.
4. Place the slides on a hot plate or slide warmer at a temperature between 65°C to 75°C for at least 2 hours (longer time is preferable), to heat-fix the samples. Do not expose slides to UV light.
5. Work systematically through the samples with slides on one side and the discard bucket in close proximity (often best at back of cabinet). Remember to open only one specimen tube at a time. Dispose of the transfer pipette into the biohazard discard bucket.

Ideally, remove smears from BSC after heat-fixation for staining.

8.2 Preparation of Smears from Positive Cultures

1. Label frosted end of slide in pencil with laboratory accession number, screening and/or subject ID number, sputum specimen number (if applicable), and date.
2. Work in a BSC as described above.

3. If examining a positive MGIT culture: vortex tube well, unscrew MGIT tube cap and sample an aliquot of broth using a disposable pipette. Place ~100 µl (2 drops) of broth onto the slide, spreading it to cover an area approximately 1 x 2 cm. Dispose of transfer pipette into the biohazard discard bucket.

4. If examining colonies on solid medium, dispense ~100 µl of distilled water on a glass slide with a transfer pipette. Using a sterile loop or disposable applicator stick, transfer 2 to 3 colonies to the water and gently mix to make a smooth, thin suspension. Dispose of applicator stick into the biohazard discard bucket.

5. Air-dry smear.

6. Place the slides on a hot plate or slide warmer at a temperature between 65°C to 75°C for at least 2 hours (longer time is preferable), to heat-fix the samples.

Ideally, remove smears from BSC after heat-fixation for staining.

8.3 Procedure for Fluorescent Staining

8.3.1 Auramine or Auramine/Rhodamine Procedure

NOTE: The procedures below should be strictly followed, unless a commercial staining kit is used, in which case the manufacturer's instructions should be followed exactly as written in the package insert.

1. Place slides on staining rack so they are at least 1 cm apart, and flood with auramine or auramine/rhodamine stain and let stand for 20 min.

2. Rinse the stain away with distilled water and tilt slide to drain. Water must be chlorine free.

3. Flood the slide with 0.5% acid alcohol and let stand for 2 min.

4. Wash off the acid alcohol with distilled water.

5. Flood slides with 0.5% potassium permanganate for 1-2 min. Do not allow potassium permanganate to act over 2 min, or it might quench the fluorescence of acid-fast bacilli.

6. Wash off the stain with distilled water.

7. Allow slides to air dry in the slide rack. DO NOT BLOT!

8. Protect smears from light and examine immediately using the fluorescent microscope. If unable to read right away, place slides in covered box.

8.3.2 Acridine Orange Procedure

1. Place slides on staining rack so they are at least 1 cm apart, flood with 2N H_2SO_4, and let stand for 10 min.

2. Wash off the 2N H2SO4 with distilled water.

3. Flood slides with acridine orange working solution and let stand for 10 min.

4. Wash off the stain with distilled water and tilt slide to drain.

5. Flood the slide with 2% acid alcohol and let stand for no more than 3 min.

6. Wash off the acid alcohol with distilled water.

7. Allow slides to air dry in the slide rack. DO NOT BLOT!

8. Protect smears from light and examine immediately using the fluorescent microscope. If unable to read right away, place slides in covered box.

8.4 Procedure for ZN Staining

1. Place slides on staining rack so they are at least 1 cm apart, and flood with carbol fuchsin.
2. Heat the slide to steaming with the flame from a Bunsen burner. An electric heating block may also be used. Apply only enough additional heat to keep the slide steaming for 5 minutes. Do not let the stain boil or dry. Add additional stain if necessary.
3. Wash off the stain with distilled water.
4. Flood slides with 3% acid-alcohol.
5. Let stand for 2-3 min (more acid-alcohol should be used if the smear is heavily stained).
6. Wash off the acid-alcohol with distilled water and tilt the slides to drain.
7. Flood the slides with methylene blue and let stand for 1-2 minute.
8. Wash off the methylene blue with distilled water.
9. Tilt the slides to drain.
10. Allow slides to air dry in the slide rack. DO NOT BLOT!

NOTES:

- Heat fixing (slide warmer or flame) does not always kill mycobacteria; exercise care when handling slides.
- Slides must not touch each other when placed on staining rack to prevent transfer of material from one slide to another.
- Fluorochrome stained smears lose fluorescence with time and exposure to light. **Keep smears covered and examine within 24 hours of staining.**

8.5 Internal Quality Control

1. Record lot numbers, expiry dates, etc. for the staining reagents on the Daily AFB Staining QC Form (Appendix C).
2. Positive and negative control slides must be included with every batch of patient slides, and when new lots of stains are received. Record these results on the Reagent/Media QC Form (Appendix E).

See Section 16.3.1: Acid Fast Stains for more details.

8.6 Storage of Slides

1. Store all fluorescent-stained sputum smears in a slide box until the end of the study.
2. Label these slides with study label and sample details after staining and smear is dry.

9 ACID-FAST BACILLI MICROSCOPY (AFB) EXAMINATION

Purpose

The purpose of AFB microscopy is to detect acid-fast bacilli in clinical specimens and cultures. Both viable and non-viable bacilli will stain and be counted.

The results of examination of stained smears are reported in a standardized way so that results can be compared and used in a manner that is relevant to patient care. The commonly used scoring systems are published by WHO/IUATLD and CDC-USA. Reporting must be according to the WHO/IUATLD system.

Principle

With Auramine O stain, organisms fluoresce bright yellow, non-specific debris stains pale yellow, and the background is almost black. With Auramine/Rhodamine stain, organisms fluoresce yellow-red in an almost black background. With Acridine Orange, organisms fluoresce red-orange in a black background.

The Ziehl-Neelsen stains acid-fast organisms red and the background debris stains blue.

All mycobacteria are acid fast, and other genera, such as *Nocardia* and *Corynebacterium* may be partially acid fast, so microscopy cannot be used to determine individual species, including *M. tuberculosis*.

A positive smear is approximately 10^4 bacilli per ml or greater.

Procedure

Materials

- Light (bright field) microscope for Ziehl-Neelsen stain
- Fluorescent microscope for fluorescent stain
- Immersion oil for Ziehl-Neelsen stain
- Slide holder
- Slide storage box
- Lens paper
- Lens cleaning solution
- Dark room (for examination under fluorescence)

Forms

- Laboratory Specimen Requisition Form – this is a site-specific form used for routine laboratory requests.
- Laboratory worksheet/workbook – this is a site-specific form used for initial recording of smear results.
- Appendix C - Daily AFB Staining QC Form
- Appendix B - Study Source Document Worksheet

9.1 Examination of Fluorescent Stain

Examine fluorescent-stained slides within 24 hours of staining.
Save all sputum smears in slide boxes.

1. With a fluorescent microscope, scan the entire smear with the 20X objective (with 10X eyepiece for a total of 200X magnification).
2. Using 20X magnification, one 2 cm length is equivalent to 30 fields, which is sufficient to report a negative result.
3. Occasionally use the 40X objective to see more detailed bacterial morphology. Confirming morphology at higher magnification avoids a false-positive report due to fluorescing debris.
4. Mycobacteria appear as rod-shaped, coccoid, or filamentous bacilli. They may be vary in shape and staining intensity, and may occur singly, in small groups containing a few bacilli, or as large clumps.
5. Count bacilli in the number of fields appropriate for the degree of positivity (e.g., the higher the smear positivity, the fewer fields counted). Average the count for the number of fields and record on the lab worksheet the appropriate result according to the table.

Table 9.1 Grading Scale for Fluorescent Stain

What you see (200x)	What you see (400x)	What to report*
No AFB in one length	No AFB in one length	No AFB observed
1-4 AFB in one length	1-2 AFB in one length	Confirmation required**
5-49 AFB in one length	3-24 AFB in one length	Scanty
3-24 AFB in one field	1-6 AFB in one field	1+
25-250 AFB in one field	7-60 AFB in one field	2+
> 250 AFB in one field	> 60 AFB in one field	3+

*The number of AFB indicates how infectious the patient is. It is important to record exactly what you see.
** Confirmation required by another technician or prepare another smear, stain and read.

9.2 Examination of Ziehl-Neelsen Stain

1. Using a bright field microscope, Ziehl-Neelsen smears are examined with the 100X oil objective (10X eye piece for a total of 1000X magnification). Take care not to touch the slide with the tip of the dropper when dispensing oil. Always wipe oil from the oil immersion lens after each AFB-positive smear is read.
2. AFB will have similar morphology as fluorescence-stained bacilli. They are variable in shape, from very short rods to long filaments. Often they are bent, contain heavily stained beads, and may be aggregated side by side and end to end to form cords, especially when grown in liquid culture (MGIT). The AFB appear bright red against the background material counterstained blue.

9.3 Reporting Smear Results

If QC smear results are acceptable, patient smear results can be reported. Smear results of concentrated sputum specimens and positive cultures are reported on the laboratory worksheet and transferred to Appendix B.

9.4 Internal Quality Control

A positive and negative control smear must be included in each batch of patient tests and with new lots of reagents. See **Section 16**: Quality Assurance for further details.

9.5 Study Data Reporting

Record the type of smear and the smear results of both concentrated sputum specimens and positive cultures on Appendix B.

Fluorescent Stain: Refer to the study reporting scheme in the table in **Section** 9.1 above.

ZN Stain: Smear results will be recorded as "positive" for acid-fast bacilli, or "negative" (no AFB seen).

9.6 Detecting Source of False AFB Smear Results

Condition	Cause	Corrective Action
False-positive	Old, used microscope slides retain material from previous smears.	Use only new slides.
	AFB transferred from a positive smear to a negative smear.	Use a staining rack and keep slides from touching each other; do not use staining jars.
	Food particles.	Request another specimen.
	Precipitated stains.	Use only fresh stains, without precipitates or contaminating organisms.
	AFB transferred in oil on the immersion lens or through contaminated oil bottles.	Always wipe oil from the oil immersion lens after each AFB-positive smear is read. Change oil bottles whenever there is evidence of contamination.
False-negative	Smears that are too thick, or slides that are not clean, causing material to be washed off during staining.	Proper digestion of specimen. Avoid making thick smears.
	Smear area is too large, making the smear too thin.	Apply smear to a 1 x 2 cm area.
	Nonstaining or poorly staining AFB.	Examine fluorescent stained smears within 24 hours. Protect smear from UV light, direct sunlight, over/under heating during smear fixation; store staining reagents in dark bottles; high chlorine content in rinse water will affect fluorescence stain. During staining, drain off excess rinse water between steps to avoiding diluting staining reagents.
	Incorrect slide warmer temperature	Set temperature at 65-75°C and monitor on a daily/weekly basis
	Incomplete slide reading	Search smear in a uniform manner and read suggested number of fields.

10 LIQUID CULTURE – MYCOBACTERIA GROWTH INDICATOR TUBE (MGIT)

Purpose

To amplify the number of *Mycobacterium tuberculosis* (MTB) organisms in a sample using a liquid culture media (MGIT) and to detect positive samples rapidly. To make a semi-quantitative assessment of the bacterial load by determining the time taken for culture tubes to signal positive (time to detection, TTD) in the BACTEC MGIT 960 system. The MGIT culture result, along with confirmatory ZN, BAP, and rapid ID tests, are the primary indicators of the presence of viable MTB in the sputum.

Principle

Mycobacteria multiply in a nutrient-rich medium, while contaminating bacteria are inhibited by the addition of a cocktail of antibiotics. Growth of bacteria, including mycobacteria, is indicated by fluorescence, which increases proportionally as oxygen decreases in the tube. The instrument detects this fluorescence in the medium using a UV light and complex computer algorithms.

Sputum specimens are processed (**Section** 7: Processing Sputum for Smear Microscopy and Qualitative Culture), and inoculated into 7ml MGIT tubes, which are supplemented with OADC (Growth Supplement) and a cocktail of antibiotics (PANTA). The MGIT tubes contain a fluorescent compound embedded in the base of the tube, which is sensitive to the presence of oxygen dissolved in the broth. Initially, the large amount of oxygen quenches the emissions from the compound and little fluorescence is detected. Bacteria present in the concentrated sputum specimens metabolize oxygen in the culture medium, allowing the fluorescence to be detected. Blood samples are not suitable for the MGIT system.

BACTEC MGIT 960 – Instrument overview

The BACTEC MGIT 960 instrument is capable of monitoring a total of 960, 7 ml MGIT tubes. The tubes are arranged in three continuously incubated drawers, labeled A, B, and C, each of which holds up to 320 tubes. Each drawer contains an apparatus consisting of:

Tube rack – rack in the drawer that holds the MGIT tubes

Stations – individual wells in the rack into which tubes are inserted.

The detector assembly –sits below the rack and has 16 detectors, one for each row of stations. The assembly moves from left to right and back, taking test readings for each of the 20 station columns and the calibrator tube.

Drawer status indicators – three lamps located on the front of each drawer; one indicates a positive (+), one indicates a negative (–), and one indicates a station error (!).

Barcode scanner – located at the front of the instrument; it is used to scan tube labels for specimen identification. The scanner turns on automatically.

LCD display and keypad – presents all the information needed to monitor the instrument and station status, to enter and remove tubes, set up the instrument, print reports, and perform routine instrument maintenance.

Additional details are found in the BACTEC MGIT 960 System's User's Manual, Chapters 3 and 4, which should be stored within easy access of the MGIT system. The operator of the MGIT must be familiar with this manual.

Summary of how the MGIT instrument works

The instrument continuously reads all the tubes. A row of LEDs below the tubes illuminates, activating their fluorescent sensors. Photo detectors take the readings. A test cycle of all drawers is completed every 60 minutes. Positive cultures are immediately flagged by an indicator light on the front of the drawer (and an optional audible alarm) and are displayed on the LCD screen.

When positive tubes are identified, they should be removed from the instrument for confirmation of results, and for isolation and detection of the organism.

Definition of a Growth Unit (GU)

The Growth Unit is an algorithmic measure of sensor fluorescence derived from the raw fluorescence voltage signal produced by optical integration of a MGIT tube in the 960 instrument. The MGIT 960 takes a reading every hour on the hour. An 'Instrument Positive' tube is signaled automatically via internal algorithms when the GU reaches or exceeds the cut off value of 75 units. This value is identified as a *True Positive*, and is confirmed by further tests such as ZN staining and contamination checks – see "Sampling Tubes for Further Analysis" section below.

If the MGIT signals the tube as positive and records a GU of **0** or higher *within 5 hours of incubation*, growth has occurred very rapidly and exploded past the 75 unit cut-off. Represented graphically, this growth curve would be very steep, compared to the gradual curve generated by the *True Positive*. If explosive growth has occurred, the software records a **T** in the growth column. Explosive growth usually means that the MGIT tube is contaminated. Contamination will be confirmed by a negative ZN smear and growth on blood agar (see the "Sampling Tubes for Further Analysis" section below).

The GU is not an indication of biomass within a culture tube. A MGIT tube that has been flagged as positive usually contains a biomass of approximately 10^5 to 10^6 CFU/ml. However, the tube can signal positive when the CFU is too low to yield a positive ZN smear. Thus, there is no direct correlation of biomass and GU at the time of instrument positivity.

Procedure

Materials

- MGIT tubes, 7 ml
- MGIT PANTA
- MGIT Growth Supplement
- 15 ml tube racks
- Sterile, transfer pipettes with graduations marking volume (individually wrapped)
- Sterile serological pipettes (10 or 20 ml)
- Pipette aid
- p1000 pipette (or equivalent) with sterile aerosol resistant tips
- Tuberculocidal disinfectant
- Discard bucket with biohazard bag insert, containing appropriate disinfectant
- Study labels
- Permanent marker

Forms

- Lab-specific forms for recording MGIT data
- Appendix D - Equipment Temperature Record (or use site-specific form, if available and equivalent)
- Appendix B - Study Source Document Worksheet
- MGIT Maintenance Log – this is a site-specific form

MGIT Daily Initiation Routine

All function checks must be performed DAILY every morning, preferably before unloading or loading of tubes.

Functions	Instructions
External indicator lamp verification	1. Close all drawers. 2. Press the <**maintenance**> soft key. 3. Press the <**test indicators**> soft key. 4. Press the <**test drawer indicators**> soft key. 5. All three external indicator lamps on all three drawers should light, as well as the instrument Alarm indicator. 6. Record this function check on MGIT Maintenance Log.
Drawer indicator lamp verification	1. Open drawer A. Press the <**test green LEDs**> soft key. 2. All the green LEDs at all the stations should light. If any does not, you should block the station(s). 3. Press the <**test green LEDs**> soft key again to extinguish the green LEDs. 4. Press the <**test red LEDs**> soft key. 5. All the red LEDs at all the stations should light. If any does not, you should block the station(s). Press the <**test red LEDs**> soft key again to extinguish the red LEDs. 6. Repeat steps 1-5 for Drawers B and C. 7. Record this function check on MGIT Maintenance Log.
Temperature verification	1. Check the temperature readings on the internal thermometers located in each drawer. 2. From the main status screen, press the <**temperature**> soft key to access drawer temperature readings. 3. Verify that the drawer temperature is currently within 1.5° C of the manual reading for each of the drawers. 4. Record all manual and instrument drawer temperatures on the MGIT Maintenance Log.

The instrument air filter maintenance must be performed at least **monthly**. To clean the filter:

1. Remove the faceplate by grasping it along the bottom edge at the finger holes. Gently but firmly pull straight out.
2. Remove the filter and wash in an antimicrobial disinfectant.
3. Dry thoroughly with paper towels and replace in the instrument.
4. The cut-out in the faceplate should surround the on/off switch. Firmly press in towards the instrument. The faceplate will snap into place.
5. Record this maintenance on the MGIT Maintenance Log on the day it was performed.

10.1 Inoculation of MGIT Tubes

Prior to use, examine all MGIT tubes for evidence of contamination or damage, as these are unsuitable for use. Inoculation of MGIT tubes must be done inside the biosafety cabinet with full PPE.

1. While the specimens are being processed (**Section** 7: Processing Sputum for Smear Microscopy and Qualitative Culture), prepare the antibiotic supplement (PANTA) for the MGIT tubes. Reconstitute MGIT PANTA with 15 ml of MGIT Growth Supplement. This mixture is stable for 5 days if stored at 2°-8°C. If the remaining mixture will be stored, label it with the contents, the date of preparation, date of expiry, and preparer initials.

2. Label the side of each MGIT tube using the study label that contains identifying information described in **Section** 6.2: Login of Sputum Specimens. Record the MGIT tube number (from the barcode label) on the lab MGIT worksheet.
3. Once dissolved, add 0.8 ml of the PANTA/growth supplement mixture to each MGIT tube using a repeat pipettor if available, or a micropipettor with sterile tip, taking care not to contaminate the tubes.
4. Using a sterile, graduated transfer pipette, add 0.5 ml of well-mixed sputum sediment (**Section** 7: Processing Sputum for Smear Microscopy and Qualitative Culture) to the MGIT tube. Dispose of transfer pipette in biohazard bucket.
5. Tightly recap the MGIT tube and mix well by gently inverting the tube several times.

10.2 Entering Tubes in the Machine

Enter tubes into the instrument as soon as possible. Always scan the MGIT barcode first; the instrument will assign the stations.

Functions	Instructions
Accession	1. Open the desired drawer. 2. Press the <**tube entry**> soft key. 3. Place the tube in the alignment block in front of the scanner with the barcode label facing the scanner. Rotate the tube if necessary. The instrument will beep once to indicate that the tube was scanned. 4. If the tube's label is damaged, use a spare barcode label. 5. The assigned station is shown in the main body of the display (along with the scanned sequence number). In addition, the station LED of the assigned station for that tube will illuminate GREEN. 6. Carefully and completely place the tube in the appropriate position. 7. Repeat steps 1-6 for each tube. 8. When finished, close the drawer or press <**exit**> to continue with the next task.

NOTES:

- The MGIT instrument will record the date each tube was entered.
- Do not turn tubes after placing them in the station.
- Do not remove tubes unless they are positive or out-of-protocol negatives (negative at 42 days).
- Do not reassign tubes to a new station.

10.3 Incubation of MGIT Tubes

Since the MGIT system automatically and continuously incubates and monitors tubes once they are placed in a station, there is no need to remove the tubes from the instrument. Cultures remain in their stations until signaled "positive", or if no growth is detected after 42 days incubation, are signaled "negative". If an instrument positive tube is determined to be smear negative for either mycobacteria or contaminants, the tube may be re-entered into the instrument, but within 5 hours of removal. If the tube is returned to the instrument (via the <**tube entry**> operation described above), positivity routines are reset, the start of incubation date is retained, and monitoring of the tube resumes. If the tube is not returned within the 5-hour re-entry window, the associated data is removed from the instrument's database and the tube will be monitored as a newly entered tube.

10.4 Dealing with Negative Tubes

Negative cultures exist as ongoing negatives while they are in the routine 42-day incubation period. A count of the number of negative cultures appears for each drawer in the summary region of the main screen menu, next to the filled circle icon. When no growth is detected after 42 days, the negative tube indicator, marked with a minus sign (-) and located in the center of the three drawer indicators, illuminates GREEN. The indicator remains lit until all negative tubes are removed through the <**remove negative tubes**> operation. Tubes can be removed via "batch" or "single tube" operation.

Functions	Instructions
Remove negatives – batch (removal of all final negative cultures from the instrument without having to scan the individual tube barcodes)	1. Open drawer where the GREEN light is on. 2. Press <**remove negative tubes**> soft key. 3. Press <**remove negative-batch**> soft key. The barcode scanner turns off, and tubes cannot be scanned. 4. Stations with negative tubes light up with flashing GREEN lights. Remove all tubes in the flashing green stations **prior** to closing the drawer. If negative tubes remain in the drawer after it is closed, the instrument will register these tubes as newly entered tubes. Start at front of drawer and remove in sequential order placing in same order in rack. 5. When all negatives are removed, press the <**ok**> soft key. 6. When all negatives have been removed, the instrument beeps 3 times, the GREEN drawer indicator light extinguishes, the barcode scanner turns off, and the <**ok**> icon appears on the display screen. 7. Close the drawer gently or press <**exit**> to continue with the next task. Repeat the steps for other drawers indicating negative tubes.
Remove negatives – single tube (each negative tube removed must be scanned by the barcode scanner)	1. Follow steps 1-2, as above. 2. Stations with negative tubes light up with flashing GREEN lights and the barcode scanner turns on. 3. As each negative tube is removed, the barcode label should be scanned prior to removing the next tube. The instrument will beep, indicating that the correct tube was scanned. The LED at the empty station extinguishes. 4. Place the tube in a rack in the sequential order of removal. 5. Continue to remove desired tubes and scan their barcode labels. 6. Follow steps 6-7 above.
Print Unloaded Negative Tubes Report	1. Press <**print report**> soft key and select <**unloaded negative tubes**> soft key. 2. When report finishes printing, compare unloaded tubes with report. Resolve any discrepancies. If tubes match report, press the <**ok**> soft key. **When you press the <ok> soft key, the information contained in the report is removed from the database.**

NOTES:

- Visually inspect all negative tubes prior to disposal. Any tubes that appear to have growth must be processed according to **Section** 10.6 below. In addition, refer to Flow Chart 1 for further instructions, and notify the Sponsor of this occurrence.
- Printouts of results of all negative tubes must be kept in the study binder.
- Autoclave all MGIT tubes prior to disposal.

10.5 Dealing with Positive Tubes

The positive tube indicator is the leftmost of the three drawer indicators. It is marked with a plus (**+**) sign, and illuminates **red** to inform you that one or more positive tubes are present in the drawer. The indicator remains lit until all positive tubes are removed through the <**Remove Positive Tubes**> operation. In addition, an optional audible alarm sounds when a newly positive tube is identified.

Functions	Instructions
Remove positives	1. Press <**silence alarm**> to mute the audible alarm (if alarm is activated). 2. Open the drawer with the illuminated RED positive light. 3. Press the <**remove positive tubes**> soft key. 4. All positive tubes will be indicated by flashing GREEN and RED lights. 5. Remove one tube at a time. Scan the positive tube's barcode label by placing the tube in the alignment block in front of the scanner with the barcode label facing the scanner. Rotate the tube if necessary. 6. The GREEN and RED lights will extinguish. 7. Place the tube into a rack or carrier to transport after removal. 8. Repeat steps 4 to 7 to remove additional positive tubes, placing them in sequential order in the rack. 9. When all positive tubes have been removed, the instrument will beep 3 times, the drawer indicator light extinguishes, the barcode scanner turns off, and the <**ok**> icon appears on the display screen.
Print Unloaded Positive Tubes Report	1. Press <**print report**> soft key and select <**unloaded positive tubes**> soft key. 2. When report finishes printing, compare unloaded tubes with report. Resolve any discrepancies. If tubes match report, press the <**ok**> soft key. **When you press the <ok> soft key, the information contained in the report is removed from the database (if the tube is not re-entered within 5 hours).**

NOTES:

- The MGIT instrument will record the date the tube signaled positive and the number of days/ hours taken to reach positivity (TTD).
- Place printouts of results of all positive tubes in the study binder.
- Stain all instrument positive tubes for AFB and subculture to a blood agar plate (BAP) upon removal from the instrument (see procedures in **Section** 10.6. below).
- In the unlikely event of a broken tube in the instrument – close the drawer and turn off the instrument. Vacate the room and follow the laboratory's SOP for actions following a spill.

10.6 Sampling Tubes for Further Analysis

Tubes that signal positive in the MGIT instrument require further analysis to determine the type of growth. The tubes should be observed visually. MTB growth appears granular and not very turbid, while contaminating bacterial growth appears turbid. A ZN smear is prepared to determine the presence/absence of acid-fast bacilli in the tube. A blood agar plate (BAP) is inoculated with the tube broth to determine the presence/absence of contaminants.

Materials

- Discard bucket with biohazard bag insert, containing tuberculocidal disinfectant
- Sterile, transfer pipettes with graduations marking volume (individually wrapped)
- Blood Agar Plates (TSA base)
- Sterile loop or disposable applicator stick
- Ziehl-Neelsen stain (carbol fuchsin, 3% acid alcohol, methylene blue)
- Permanent marker
- Microscope slide, with frosted end, new and clean
- Pencil for labeling slide
- Wax pencil for encircling smears on slide
- Lowenstein Jensen (LJ) slant

Work with positive MGIT tubes must be done inside the biosafety cabinet, using full PPE.

1. If multiple positive tubes are being worked up, smears from two specimens can be examined on one slide (as long as they can be spaced sufficiently so as not to cause interfering results). Blood agar plates can be divided into 4 quadrants so that four specimens can be subcultured onto one plate. Label glass slide and blood agar plate accordingly with lab accession numbers, screening and/or subject ID numbers, visit numbers, sputum specimen number, and date. **Take care not to confuse the inoculations and labeling.**

2. Vortex the MGIT tube well, unscrew tube cap and sample an aliquot of broth using a sterile, disposable pipette. Remove about 200 µl of broth (~ 4 drops) and inoculate the blood agar plate with 2 drops, streaking with the pipette tip. Use the same pipette to place 2 drops of broth on a slide. Use wax pencil or slides with etched circles to help contain smear contents. Dispose of transfer pipette into the biohazard discard bucket.

3. Re-enter the MGIT tube into the instrument within 5 hours of removal, to ensure the original culture results are retained in the instrument database.

4. Heat-fix the slide for Ziehl-Neelsen (ZN) staining as described in **Section** 8: Acid-fast Bacilli Microscopy (AFB) Preparation and Staining. Examine the ZN smear for the presence of acid-fast bacteria (AFB) as described in **Section** 9: Acid-fast Bacilli Microscopy (AFB) Examination.

If AFB are seen, describe them as typical, atypical, and note whether cording is seen. Record the description and smear result on the laboratory worksheet.

5. Incubate the blood agar plate in the incubator at 35°C (±1°C) for 72 hours (if 35°C incubation is not available, incubate at 37°C. The lower temperature is preferred as it facilitates the growth of contaminants).

10.7 Interpreting MGIT Results

Below are the possible results that can be obtained from the ZN/BAP testing. *Beneath* each table are the subsequent steps to take to determine a final result for the MGIT culture. All results and further testing/steps should be documented in the lab worksheets/notebook. **Please refer to Flow Charts 1-3 for additional guidance.**

ZN Result	BAP Result	TTD
Negative	Growth	≥ 7 days

1. This tube is contaminated; it can be discarded.

ZN Result	BAP Result	TTD
Negative	Growth	< 7 days

2. Re-incubate the MGIT tube for another 14 days and repeat the ZN smear and BAP. This ensures that low numbers of *M. tuberculosis* do not go undetected.
   a. If the subsequent ZN is positive, follow step **3** below.
   b. If the subsequent ZN is negative, this tube is confirmed as contaminated and can be discarded. Record the results from initial/subsequent testing and reincubation of this culture on Appendix M: Early MGIT Positive/Early Contaminated Cultures - Tracking Worksheet.

ZN Result	BAP Result	TTD
Positive	Growth	Any

3. Perform a ZN smear from the BAP growth to determine if rapidly growing mycobacteria are the source of contamination. In addition, perform a MPT/MPB 64 antigen test from the MGIT broth to confirm the presence of MTB. If necessary, one attempt should be made to purify the culture (see **Section** 10.10 below), if the contamination interferes with the antigen test.
   a. If the ZN from the BAP is positive and the ID test is negative for MTB, reincubate the MGIT culture for 48 hours and retest with the MPT/MPB 64 antigen test. Document the reincubation and repeat testing in the "Comments" section of Appendix B.
      i. If the ID test is now positive for MTB, this culture is resulted as "Positive for MTB complex and contaminated".
      ii. If the ID test is still negative, perform the HAIN GenoType® Mycobacterium CM or HAIN GenoType® MTBDRplus tests, following the package insert instructions.
         1. If either test is positive for MTB complex, the culture is resulted as "Positive for MTB complex and contaminated."
         2. If the GenoType® Mycobacterium CM test is positive for a non-tuberculous mycobacteria, or if the MTBDRplus test is negative for MTB complex, the culture is resulted as "No *M. tuberculosis* complex growth but positive for other mycobacteria."
   b. If the ZN from the BAP is negative, use the results from the rapid ID test to result the MGIT culture:
      i. If rapid test is positive for MTB, the culture is "Positive for *M. tuberculosis* and contaminated."
      ii. If rapid ID test is negative for MTB, follow the steps in 3a above for reincubation and retesting.

ZN Result	BAP Result	TTD
Positive	No growth	Any

4. Perform a MPT/MTB 64 antigen test to confirm the presence of MTB.

   a. If MTB is confirmed, record the culture result as "Positive for *M. tuberculosis* complex".

   b. If the test is negative or invalid, reincubate for 48 hours and retest with the MPT/MPB 64 antigen test. Document the reincubation and repeat testing in the "Comments" section of Appendix B.

      i. If the ID test is now positive for MTB, this culture is resulted as "Positive for MTB complex."

      ii. If the ID test is still negative, perform the HAIN GenoType® Mycobacterium CM or HAIN GenoType® MTBDRplus tests, following the package insert instructions.

         1. If either test is positive for MTB complex, the culture is resulted as "Positive for MTB complex."

         2. If the GenoType® Mycobacterium CM test is positive for a non-tuberculous mycobacteria, or if the MTBDRplus test is negative for MTB complex,, the culture is resulted as "No *M. tuberculosis* complex growth but positive for other mycobacteria."

ZN Result	BAP Result	TTD
Negative	No growth	Any

5. Although rare, this situation does occur and requires further investigation. Re-incubate the MGIT tube for at least another 3 days (start counting from the date of the original positive signal), or until the MGIT signals positive again. Repeat the ZN smear and BAP, taking note of the original TTD and date of positive MGIT result. Record the results from the initial/subsequent testing, including original date of positivity and TTD, results from each ZN/BAP test, whether the culture was reincubated, location of incubation, total number of days reincubated (and TTD from MGIT printout, if applicable), date of MGIT result at 42 days (if applicable), and final results of this culture, on Appendix M: Early MGIT Positive/Early Contaminated Cultures Worksheet.

   a. If the subsequent ZN is positive and BAP negative, proceed as in step **4** above, taking note of the additional days/hours of incubation to report a final TTD on the lab worksheet and Appendix B.

   b. If the subsequent ZN is positive and BAP is contaminated, proceed as in step **3** above, taking note of the additional days/hours of incubation to report a final TTD on the lab worksheet and Appendix B.

   c. If the subsequent ZN smear is still negative and the BAP is contaminated, proceed as in step **2** above, regardless of the original TTD for this culture.

   d. If the subsequent ZN smear is still negative and the BAP still has no growth, re-incubate the MGIT tube (either in the MGIT machine if within 5 hours of removal, or in a 37°C [±1°C] incubator if more than 5 hours have passed since removal) for the full 42-day protocol. If the machine signals positive again prior to the end of the protocol, repeat the ZN and BAP tests, and proceed as specified in step **1**, **3**, or **4** above. If still negative, re-incubate offline for the remainder of the 42-day protocol.

   e. Repeat the ZN and BAP tests after the 42 day incubation period, and proceed as in steps **1**, **3**, or **4** above.

      i. If after 42 days, the ZN is negative and the BAP has no growth, visually inspect the tube for signs of growth (e.g. turbidity), and test the MGIT broth with an MPT/MTB 64 antigen test. Document whether or not the tube is turbid and the result of the identification test on Appendix M.

ii. In addition, subculture the MGIT broth to a LJ slant (**Section** 11: Solid Culture: Lowenstein Jensen (LJ) Media) and incubate both the LJ and MGIT at 37°C (±1°C) in an incubator. Record the LJ subculture date on Appendix M.

iii. Notify the Sponsor **immediately** for further assistance on how to report this culture result, and document the date of notification on Appendix M.

6. In rare cases, non-acid fast bacteria may be seen on a ZN negative smear, while the BAP has no growth at 72 hours of incubation. Re-incubate the BAP for an additional 24 hours and repeat the ZN smear from the MGIT. If the same organism is again identified on the smear, **and verified by a second technician**, and the 96-hour BAP remains no growth, note the presence and type of non-acid fast organisms on the laboratory worksheet. In addition, consult the lab supervisor and the Sponsor **immediately** for further assistance on how to report this culture result.

10.8 Drug Susceptibility Testing from MGIT Cultures

1. MTB growth from the MGIT tube will be used for drug susceptibility testing at specified timepoints, following the guidelines outlined in **Section** 12: Drug Susceptibility Testing: MGIT System.

2. If a MGIT culture is resulted as "Positive for MTB complex and contaminated", a DST is required (see Section 2: Sample Specimen Timetable), and the primary LJ culture is unavailable for testing, one attempt should be made to recover *M. tuberculosis*. (see Section 10.10 below).

10.9 Subculture and Storage of MGIT Cultures

1. Vortex the MGIT tube well and remove tube cap. Using a disposable pipette, remove a small aliquot (~200 ul) of broth and inoculate 1-2 LJ tube(s); incubate at 37°C (±1°C) (**Section** 11: Solid Culture: Lowenstein Jensen (LJ) Media). Growth from this LJ subculture can be used for drug susceptibility testing, if this test is unsuccessful using the MGIT broth. This LJ subculture is used for the short-term and long-term storage of isolates (**Section** 11: Solid Culture: Lowenstein Jensen (LJ) and **Section** 14: Long-Term Storage of MTB Isolates).

2. Store all instrument-positive MGIT tubes, including cultures growing MTB or MOTT, for at least 3 months after the culture is finalized, at room temperature away from direct light.

10.10 Decontamination of Contaminated MGIT Culture

Decontamination of a MGIT tube should be performed in the following situations:

- When the culture result is "Positive for MTB and contaminated", and the specimen is from a timepoint that requires DST, and the corresponding primary LJ culture is not positive for MTB.
- If the antigen test result is indeterminate, owing to the presence of contaminants.
- To prepare the short-term LJ storage and subsequent long-term frozen isolate, if the corresponding primary LJ culture is not positive for MTB.

1. Vortex contaminated MGIT tube; let stand for about 1 minute.
2. Aseptically transfer entire volume of broth into a 50 ml disposable centrifuge tube.
3. Add an equal quantity of 4% NaOH solution, for a final concentration of 2% NaOH.
4. Mix well and let stand for 15-20 minutes, mixing and inverting the tube periodically.
5. Add phosphate buffer, pH 6.8 to the 50 ml mark and mix well.

6. Centrifuge at 3,000 *xg* for 15-20 minutes.
7. Pour off the supernatant fluid.
8. Resuspend the sediment in 0.5 ml of phosphate buffer and mix well.
9. Inoculate 0.5 ml into a fresh MGIT tube supplemented with MGIT growth supplement/PANTA according to Section 10.1 above, and reincubate following instructions in **Section** 10.2 and 10.3.

10.11 Reporting Results of MGIT Cultures

Mycobacterial growth must be reported immediately on the laboratory worksheet. Use the following table to report the result of the MGIT culture on Appendix B.

MGIT Machine Result	ZN Result	BAP Result	ID Result	Appendix B Result	Time to Result (TTD) days/hours*
Positive	Positive	No growth	MTB complex detected by rapid ID test, HAIN GenoType® Mycobacterium CM, or HAIN GenoType® MTBDRplus tests	Positive for *M. tuberculosis* complex, ID positive for MTB complex; record ID method	Original TTD from MGIT printout (and final TTD from Appendix M, if applicable)
Positive	Positive	Growth	MTB complex detected by rapid ID test, HAIN GenoType® Mycobacterium CM, or HAIN GenoType® MTBDRplus tests	Positive for *M. tuberculosis* complex and contaminated, ID positive for MTB complex; record ID method	Original TTD from MGIT printout (and final TTD from Appendix M, if applicable)
Positive	Positive	Negative or growth	MTB not detected by HAIN GenoType® MTBDRplus test, or MOTT detected by HAIN GenoType® Mycobacterium CM test	No *M. tuberculosis* complex growth but positive for other mycobacteria; ID negative for MTB complex; record ID method	Original TTD from MGIT printout (and final TTD from Appendix M, if applicable)
Positive	Negative	Growth	N/A	Contaminated	Original TTD from MGIT printout (and final TTD from Appendix M, if applicable)
Negative	N/A	N/A	N/A	Negative for *M. tuberculosis* complex	Original TTD from MGIT printout, and a final TTD of 42 days, 0 hours

* Refer to Section 10.14 Study Data Reporting for additional instructions for recording the TTD.

NOTE:

If MGIT culture signals negative, but tube appears turbid when removed from the instrument, process according to the guidelines in Section 10.6 and Flow Chart 1, notifying the Sponsor of this occurrence.

10.12 Quality Control of MGIT Media

1. Record lot number, expiry dates, etc. for new MGIT medium and supplement/ PANTA on the Reagent/Media QC form, Appendix E.
2. Quality control procedures for MGIT media, and for the evaluation of TTD reproducibility, are described in **Section** 16: Quality Assurance.

10.13 Quality Control/Quality Monitoring of MGIT Instrument

Perform daily functional and temperature checks of the MGIT instrument and record on the MGIT Maintenance Log. Each month, run the MGIT QC Report (a report generated by the MGIT 960 instrument), which lists the status of all the detectors in the instrument, along with the date and time of their last verification. The report also lists all manually blocked stations. Print out the report and maintain in the laboratory files.

10.14 Study Data Reporting

All liquid culture data (date of inoculation, date of MGIT result, culture result, original time-to-detection in days and hours from the MGIT printout, final time-to-detection in days and hours (the final TTD would only differ from the original if reincubation was required and Appendix M completed, or if a negative culture was incubated longer than 42 days, 0 hours), ZN, BAP, and ID results, final date of MGIT culture completion, and any re-decontamination) are reported on Appendix B. Culture results and TTD are reported according to the guidelines listed in section 10.11 above.

NOTES

1. "Date of MGIT result" on Appendix B should be the date the actual culture result was determined. This will be the date on the MGIT printout from the original incubation of the culture, or if the culture was reincubated, this date represents the date when the final culture result was obtained and final TTD calculated from Appendix M.

2. "Final date of MGIT culture completion" on Appendix B should be the date all testing in the MGIT section is complete (i.e. ZN, BAP, and ID).

3. If ZN, BAP, and ID are not performed because culture is negative, mark "N/A" for each of these tests on Appendix B.

4. If the MGIT culture is determined to be "Positive for *M. tuberculosis* complex", and the original ZN was negative (culture was re-incubated and subsequent ZN was positive for acid-fast bacilli), two TTDs will be reported: 1)the original TTD from the MGIT printout, and 2) the revised, final TTD as reported on Appendix M.

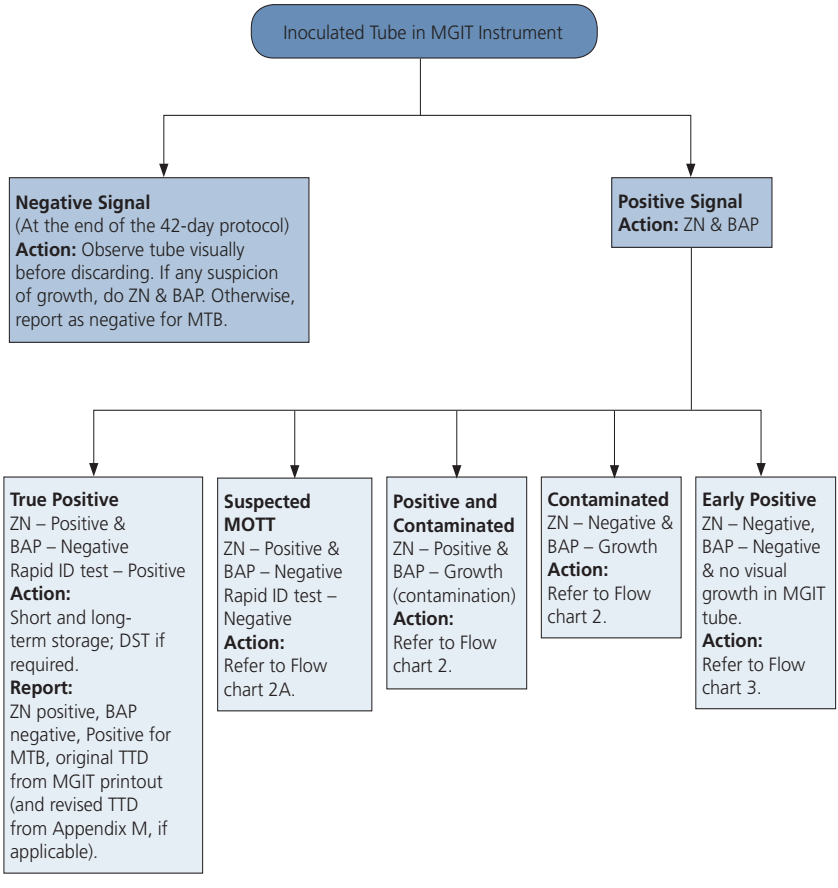
5. If a "Positive for *M. tuberculosis* complex and contaminated" culture is re-decontaminated to perform ID, DST, etc., record the MGIT culture result, the TTD, and the date of MGIT result from the **original** sputum culture on the lab worksheet and Appendix B. It should also be noted on Appendix B that the MGIT tube was decontaminated and re-cultured. If unable to successfully recover MTB from a "Positive for *M. tuberculosis* complex and contaminated" culture, document this fact in the MGIT "Comments" field on Appendix B.

6. If the MGIT culture is determined to be "Contaminated", record both the original TTD from the MGIT printout, and the final TTD as recorded on Appendix M (if reincubation was performed).

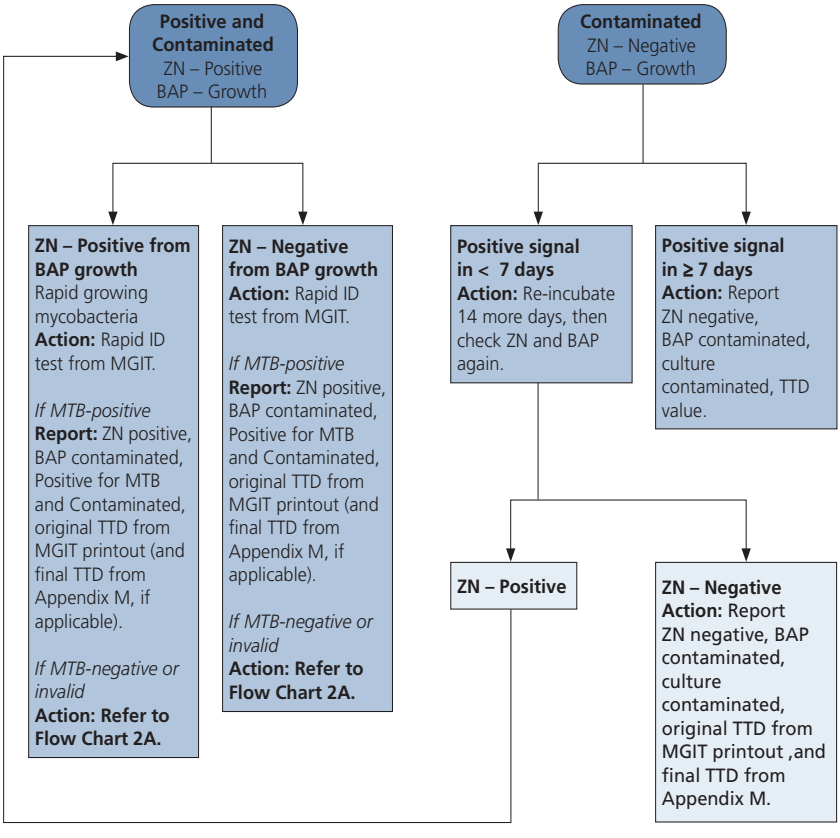
7. If the MGIT culture is determined to be "Negative for *M. tuberculosis* Complex", record the "TIP" value reported on the MGIT "unloaded negatives" printout as the original TTD on Appendix B. The final TTD should be written as "42 days, 0 hours" on Appendix B.

8. In the unlikely event that a culture result cannot be obtained (e.g., broken or lost MGIT tube, MGIT instrument malfunction, etc.), report the MGIT culture result as "Unknown" and provide a brief explanation in the MGIT "Comments" field on Appendix B. In addition, notify the Sponsor of any cultures reported as "Unknown."

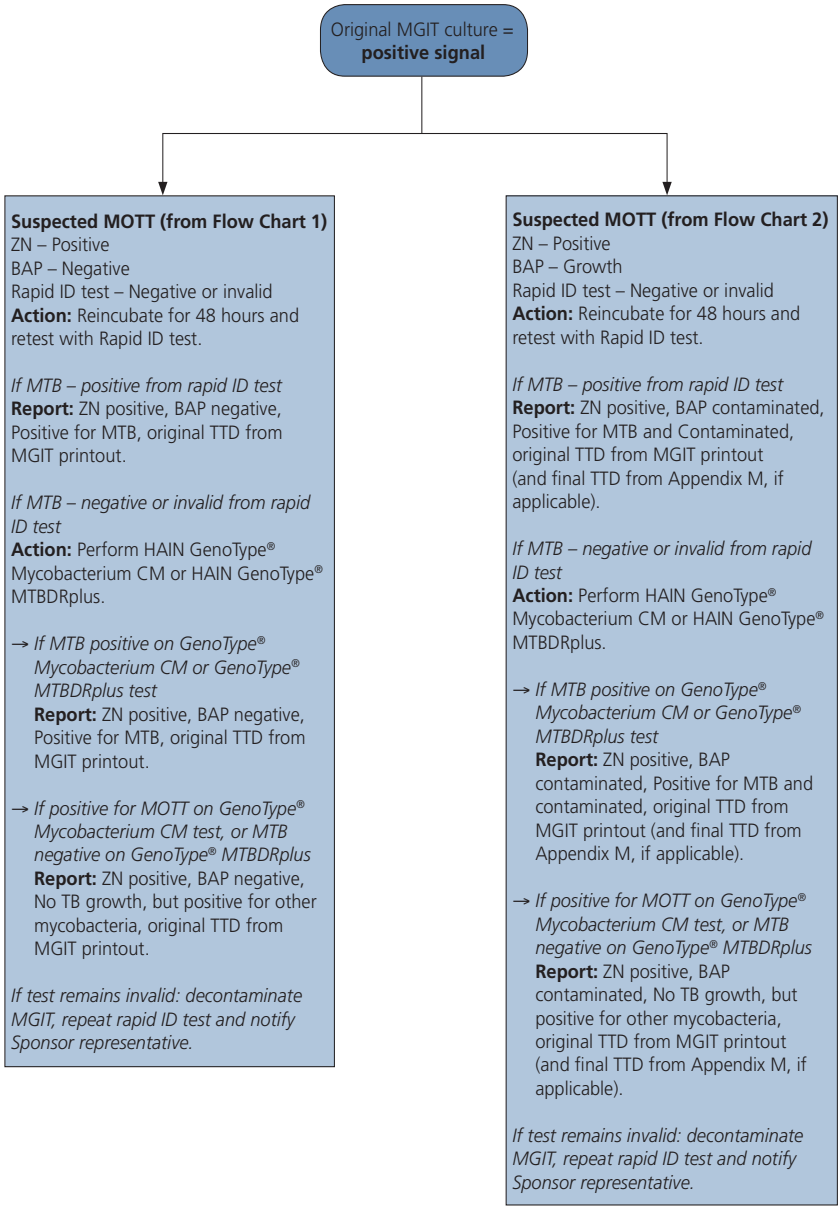
Flow Chart 1: General Algorithm MGIT 960 Cultures



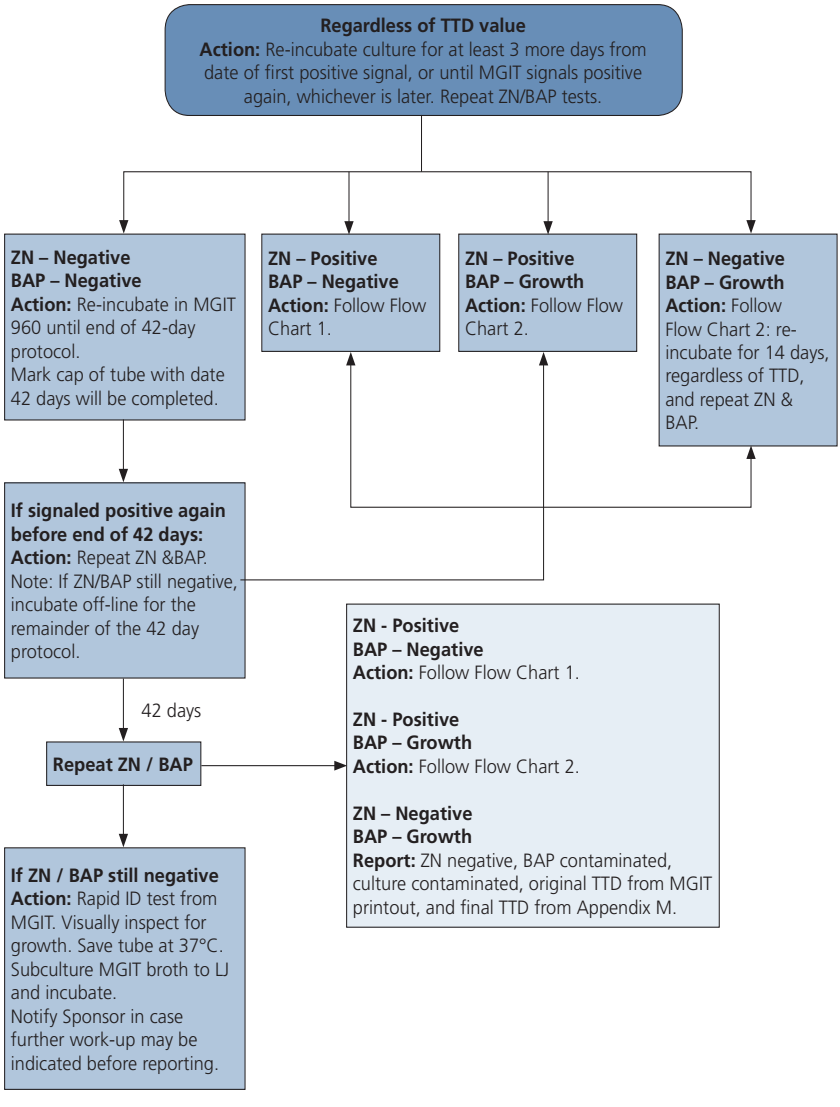
Flow Chart 2: Contaminated MGIT Cultures



Flow Chart 2A: Suspected MOTT Cultures



Flow Chart 3: MGIT "Early Positive" Cultures (ZN/BAP negative)



11 SOLID CULTURE: LOWENSTEIN JENSEN (LJ) MEDIA

Purpose

The purpose of this procedure is to isolate and semi-quantify growth of *M. tuberculosis* on LJ medium. Slants will be inoculated with decontaminated and concentrated sputum specimens (**Section** 7: Processing Sputum for Smear Microscopy and Qualitative Culture). Slants will also be inoculated from each positive MGIT tube. Once good growth is obtained, these positive slants will be stored in a cool, dark place to archive the positive *M. tuberculosis* isolates.

Principle

Many different solid media are available for cultivating mycobacteria. Most are variations of egg-potato base or albumin-agar base media. There is no general consensus on which medium is best for routine isolation. The advantages of egg-based media such as LJ are: 1) it is easy and economical to prepare, 2) it is associated with lower contamination rates, and 3) isolated colonies with characteristic colony morphology for MTB can be observed. Disadvantages are: 1) when contamination occurs, it often involves total surface of medium, 2) if contamination is slight, it is not evident when mycobacterial growth is confluent, and 3) drug susceptibility tests are more difficult to perform using egg-based media because some drugs must be adjusted to account for their loss by heating or by interaction with certain components of the egg.

As with all media preparation, attention must be given to purity of chemical components, including quality of eggs; preparing and sterilizing medium and glassware; exposure of final product to excessive heat or sunlight; and method and length of storage. All lab-prepared media must be tested for sterility and performance characteristics before being used.

Procedure

Materials

- LJ medium
- Sterile, transfer pipettes with graduations marking volume (individually packaged)
- Tuberculocidal disinfectant
- Discard bucket with biohazard bag insert, containing appropriate disinfectant
- Sterile loop or disposable applicator stick
- Ziehl-Neelsen stain (carbol fuchsin, 3% acid alcohol, methylene blue)
- Parafilm
- Microscope slides, frosted at one end, new and clean
- Paper towel soaked in disinfectant
- Sterile distilled water
- Permanent marker
- Pencil for labeling slides
- Study labels
- Incubator

Forms

- Lab-specific forms for reading/recording LJ results
- Laboratory Specimen Requisition Form– this is a site-specific form used for routine laboratory requests
- Appendix B - Study Source Document Worksheet
- Appendix E - Reagent/Media QC Form
- Appendix J - Storage Log for *M. tuberculosis* Isolates on LJ Media

11.1 Inoculation and Incubation of Solid Cultures

Inoculation of slants must be done inside the biosafety cabinet, using full PPE.

1. Label LJ tube, using the study-specific labels that contain identifying information as described in **Section** 6.2: Login of Sputum Specimens.
2. Remove any excess water in the slant using a sterile transfer pipette.
3. Inoculate the tube with 200 µl of the sample (either well-mixed, processed sputum – **Section** 7: Processing Sputum for Smear Microscopy and Qualitative Culture; or positive MGIT tube – **Section** 10: Liquid Culture – Mycobacteria Growth Indicator Tube (MGIT)) using a sterile graduated disposable pipette. Spread inoculum evenly over entire surface of medium. Take care to minimize aerosol generation when sampling from positive MGIT tubes as this broth will contain large numbers of *M. tuberculosis*.
4. Replace cap and ensure there are no droplets around the rim of the tube. Wipe off the outside of the tube with a paper towel soaked in tuberculocidal disinfectant.
5. Leave tube in slanted position with cap loosened until inoculum is absorbed (about a week), then tighten cap securely and incubate in upright position at 37°C (±1°C). Alternatively, the tube can be incubated immediately in an upright position with cap loose for the first week of incubation.
6. Examine and record results for the cultures weekly, for 8 intervals. Cultures can be read on the bench, as long as the caps are NOT loosened.
7. To observe fine growth, a strong direct light from the angle poise lamp must be shone onto the slant surface. *M. tuberculosis* usually grows as a buff-colored, dry colony, which is very distinctive.

11.2 Working up Growth on Primary Solid Culture

1. To check for AFB and purity, select 2 to 3 colonies of representative growth using a sterile loop or applicator stick, make a smear and perform a ZN stain (**Section** 8: Acid-fast Bacilli Microscopy (AFB) Preparation and Staining and **Section** 9: Acid-fast Bacilli Microscopy (AFB) Examination).

   NOTE: Contamination of LJ may be obvious, and a ZN smear may either not be necessary, or not practical (e.g., when the entire slant dissolves); in these situations, mark "N/A" on Appendix B. If contamination is only present on a small surface area of the slant, continue to incubate to allow MTB to grow. If a ZN smear is performed, the results should be recorded on the lab worksheet and Appendix B.
2. Note the colony morphology and pigmentation on solid medium, and describe these features in the laboratory worksheet/workbook. Also, assess time for growth to appear on medium. Typical growth characteristics, along with Ziehl-Neelsen staining properties, are suggestive of *M. tuberculosis* complex.

3. At all visits, growth that is AFB positive requires confirmation as *M. tuberculosis* complex using the MPT/MTB 64 antigen test, and results recorded on the lab worksheet and Appendix B (Refer to **Section** 13: Rapid Identification of *M. tuberculosis* Complex for details).

4. On specific visits, growth which has been confirmed as MTB complex will be used for drug susceptibility testing, *if the corresponding MGIT culture is not usable*. (See **Section** 2: Sample Specimen Timetable and **Section** 12: Drug Susceptibility Testing: MGIT System.)

11.3 Working up Growth from MGIT Subculture on LJ Media

1. To check for AFB and purity, select 2 to 3 colonies of representative growth using a loop or applicator stick and perform a ZN stain (**Section** 8: Acid-fast Bacilli Microscopy (AFB) Preparation and Staining and **Section** 9: Acid-fast Bacilli Microscopy (AFB) Examination).

2. If growth on solid medium is contaminated or insufficient to archive, prepare another tube using growth from original LJ slant. Save the culture in the isolate storage bank (See **Section** 11.5. below).

11.4 Recording Results of Primary Solid Culture

1. Record weekly growth results on the laboratory worksheet.

2. At weeks 1 through 7 if there is no growth, record "neg". If at the 8th read date the culture is negative, record "no growth" on the laboratory worksheet.

3. If there is growth at any reading interval, re-incubate the tube and read again the following week.

    - If the same approximate count is seen, reading can be stopped and this count can be considered final.
    - However, if the count increases substantially (e.g., **1+** at week 2, **2+** at week 3) the following week, continue to read the culture weekly until growth stabilizes.

4. Use the following standardized reporting scheme to report growth from the solid culture on the laboratory worksheet and Appendix B.

Growth	Laboratory Report	ZN Result	ID Result	Study Report – Solid Culture
None	No growth	N/A	N/A	Negative for MTB complex
1-9 colonies	Record actual number	pos	MTB	TB growth (1-9 colonies); record ID result and test method
10-100 colonies	1+	pos	MTB	TB growth (10-100 colonies); record ID result and test method
> 100-200 colonies	2+	pos	MTB	TB growth (more than 100 colonies); record ID result and test method
> 200 colonies (too numerous to count or confluent)	3+	pos	MTB	TB growth (innumerable or confluent); record ID result and test method
Other mycobacterial growth	Positive for other mycobacteria	pos	neg	No MTB complex growth, but positive for other mycobacteria; record ID result and test method[1]
Contaminated	Contaminated	N/A	N/A	Contaminated
ZN+ growth in presence of contamination	Positive for MTB and contaminated	pos	MTB	Positive for MTB complex and contaminated; record ID result and test method[2]

1 If growth appears to be or resembles MOTT, and the MPT/MTB 64 antigen test is indeterminate due to the presence of contaminants, presumptively ID the culture by looking for typical growth characteristics and ZN staining properties of MOTT. Report as "No MTB complex growth, but positive for other mycobacteria".
2 If growth appears to be or resembles MTB, and the MPT/MTB 64 antigen test is indeterminate due to presence of contaminants, presumptively ID the culture by looking for typical growth characteristics and ZN staining properties of MTB complex and report as "Positive for MTB and contaminated".

NOTE:
In case of either #1 or #2 above, leave the ID test method blank and write a comment in the LJ Culture "Comments" section, such as, "Rapid ID test result indeterminate due to contamination; presumptive identification made for culture".

11.5 Logging in LJ Cultures for Isolate Storage Bank

Ensure growth is consistent with MTB prior to storing for short-term storage. If this tube is removed to conduct additional testing, a new subculture must be prepared to ensure there is always a short-term storage tube.

1. All isolates grown on the LJ subculture (from the MGIT culture) are stored in numerical order according to screening ID number and/or subject ID number, sputum specimen number, lab accession number, and visit number. These isolates should be stored at room temperature (preferred) or in the refrigerator for at least one year from the date of preparation.

2. Seal the cap of the LJ tubes with parafilm. Be sure that tubes are clearly labeled with a study label that includes identifying information as described in **Section** 6.2: Login of Sputum Specimens.

3. Login these data for each specimen on the Storage Log for *M. tuberculosis* Isolates on LJ Media (Appendix J).

4. Use these LJ subcultures to prepare the frozen isolates for long-term storage (i.e. for 6 months after the conclusion of the study). Refer to Section 14: Long-Term Storage of MTB Isolates.

5. After one year of storage, LJ subcultures can be designated for disposal, provided that all necessary testing has been completed and all frozen stock aliquots have been appropriately stored.

NOTE: Approval to discard these LJ subcultures must be given by the Sponsor prior to disposal.

11.6 Quality Control of Media

Records of batch numbers, dates of preparation, expiration dates, and QC results of all media should be documented on the Reagent/Media QC Form (Appendix E) when media is put into use. Refer to **Section** 16: Quality Assurance for details on sterility and performance testing for both laboratory-prepared and commercially prepared media.

11.7 Study Data Reporting

Use the reporting scheme in **Section** 11.4 above to record results of LJ culture growth. Record date of inoculation, date of result, ZN result, and identification of species in the "LJ Culture of Sputum" section on Appendix B. In addition, check the appropriate box on pg. 1 of Appendix B to confirm whether the isolate was archived for short-term storage.

NOTES:

1. If ZN is not performed because culture is negative, mark "N/A" for this test on Appendix B.

2. In the unlikely event that a culture result cannot be obtained (e.g., broken or lost tube), report the solid culture result as "Unknown" and provide a brief explanation in the LJ "Comments" field of Appendix B. In addition, notify the Sponsor of any cultures reported as "Unknown."

12 DRUG SUSCEPTIBILITY TESTING: MGIT SYSTEM

Purpose

Multidrug resistant (MDR) TB is caused by strains of MTB resistant to at least isoniazid and rifampicin, and effective patient management requires optimized treatment regimens. These complex regimens can include a fluoroquinolone, injectable drugs (either an aminoglycoside or a cyclic peptide), compounds from other drug classes, as well as any remaining first-line susceptible drugs. Therefore, reliable drug susceptibility testing (DST) of these anti-TB drugs is crucial for the management of MDR-TB and for preventing emergence of additional drug resistance in these patients.
DST will be performed on isolates from newly enrolled patients and at regular timepoints throughout the trial. As two specimens are collected at each timepoint, DST will be performed on the first positive culture from the set only (not both).

All first-line drugs (streptomycin, isoniazid, rifampicin, ethambutol (SIRE); and pyrazinamide (PZA)) will be tested in the MGIT 960 system. Additionally, the fluoroquinolones (gatifloxacin, levofloxacin, moxifloxacin, ofloxacin) and injectable drugs (amikacin, capreomycin, kanamycin) used in local standard of care will be tested in the MGIT 960 system, unless the MGIT method is unreliable for a specific second-line drug in MGIT. DST of these second line drugs is well-established, and results are reliable and reproducible. Therefore, they are the only DST results required for testing positive cultures from patients.

If a fluoroquinolone or injectable cannot be tested in MGIT, it is acceptable to perform DST using the proportion method on solid medium. Each lab performing solid media testing must submit their approved SOP for this procedure, and demonstrate satisfactory performance in routine quality control testing of reference strains.

While the study protocol does not require testing/reporting of other second/third-line drugs, the investigator and/or lab manager may request DST of these drugs as necessary to appropriately manage the study patients. These results will be reported on a separate, lab-specific form (not on Appendix B).

The MGIT system is recommended for testing second-line drugs because 1) the system yields results faster than the growth-based methods using a solid medium, 2) testing must be carried out according to the recommended published procedures in order for the system to be operational (providing some degree of standardization), and 3) the instrument automatically detects presence of growth and completion of test. Furthermore, WHO and other experts have published recommended critical concentrations for testing many second-line drugs for the MGIT system.

Principle

Susceptibility testing in the MGIT 960 system is based on the same principles as isolation from sputum (detection of growth). DST is performed using an AST (antibiotic susceptibility testing) set, which consists of a Growth Control tube and one tube for each drug, as well as a bar-coded tube carrier that holds the set. A known concentration of drug is added to a MGIT tube, along with the specimen, and growth is compared with a drug-free control of the same specimen. If the drug is active against the mycobacterial isolate (isolate susceptible), growth will be inhibited and fluorescence will be suppressed in the drug-containing tube; meanwhile, the drug-free control will grow and show increasing fluorescence. If the isolate is resistant, growth and its corresponding increase in fluorescence will be evident in both the drug-containing and the drug-free tube.

The MGIT 960 system monitors these growth patterns and can automatically interprets results as susceptible or resistant. An isolate is defined as resistant if 1% or more of the test population grows in the presence of the critical concentration of the drug.

BACTEC MGIT 960 – AST overview

When the AST feature is enabled in the MGIT 960 instrument, the Positive indicator on the drawer, in addition to indicating positive MGIT growth tubes in the drawer, also indicates the presence of one or more **completed** AST sets. The station indicators illuminate in the same pattern as with individual MGIT tubes. However, with AST tube sets, ALL the stations for the set illuminate or flash. The leftmost station is ALWAYS the assigned location of the set's Growth Control tube.

Additional details are found in the BACTEC MGIT 960 System's AST manual, which should be stored within easy access of the MGIT system. The operator of the MGIT must be familiar with this manual.

Procedure

Materials

- Discard bucket with biohazard bag and appropriate tuberculocidal disinfectant
- 7ml MGIT tubes
- 7ml MGIT PZA medium tubes
- BD SIRE MGIT kit reagents
- BD Pyrazinamide MGIT kit reagents
- Second-line drug powders (depending upon local standard of care recommendations):
  - Amikacin (Sigma catalog # A1774 or A2324)
  - Capreomycin (Sigma catalog # C4142)
  - Gatifloxacin (Sigma catalog # G7298)
  - Kanamycin (Sigma catalog # K1876 or K4000)
  - Levofloxacin (Sigma catalog # 28266)
  - Moxifloxacin (Sigma catalog # 32477)
  - Ofloxacin (Sigma catalog # O8757)
- BD BACTEC MGIT SIRE and PZA supplement
- MGIT OADC Enrichment (MGIT supplement for second line drugs, BD catalog # 245116)
- AST carrier sets
- Sterile distilled/deionized water
- NaOH, for dissolving Gatifloxacin and Ofloxacin, if applicable
- McFarland standards (0.5 and 1.0)
- Pipet Aid
- p1000 and p200 pipettes (or equivalent) and sterile aerosol resistant tips
- Sterile graduated serological pipettes (5 and 10 ml)

- Sterile graduated transfer pipettes, individually wrapped
- Sterile saline
- Blood agar plates
- Middlebrook 7H9 broth, for preparing inoculum from positive LJ cultures
- 16.5 x 128 mm sterile disposable tubes, or tubes of the same size (diameter) as the lab's McFarland standards
- Sterile disposable loop or applicator stick
- Capped sterile tubes containing 2 mm glass beads, for preparing inoculum from LJ
- BBL Middlebrook 7H9 broth
- Vortex
- Analytical balance and weigh boats
- Graduated cylinder
- Erlenmeyer flask or media bottles
- Sterile syringes (10 or 20 ml)
- Syringe filters (0.22 µm pore size; e.g., Millex-GS)
- 2 ml sterile cryotubes with screw top (polyethylene or polypropylene)
- BD Bactec AST Carriers
- Permanent marker
- Study labels

Forms

- Lab-specific forms for recording DST results
- Appendix F - DST QC Form (or use site-specific form, if available and equivalent)
- Appendix B - Study Source Document

12.1 Preparing AST Carriers

The susceptibility test may be configured in a variety of formats. Since different profiles of non-MGIT drugs will be tested, check the BACTEC MGIT 960 User's Manual (AST Instructions and Appendix A) for AST carrier sizes and optional configurations. When setting up the AST tube sets, you must use the correct size tube carrier for the AST set you are testing. The number of tubes in the set is encoded in the carrier's barcode. Using a carrier of the incorrect size will cause the system to interpret the absence of a tube as an error, which invalidates the results for the entire AST set.

12.2 Preparation of Drug Stocks for Susceptibility Testing

12.2.1 SIRE and PZA Drug Stock Preparation

Drug stocks and preparation of MGIT tubes must be carried out inside the biosafety cabinet, using full PPE.

Reconstitute the drugs with the appropriate volume of diluent. Volumes vary with different drugs. Failure to use the appropriate volume will invalidate these tests. Refer to Table 12.1 - Drug Concentrations for DST in MGIT, below.

Task	Instructions
Prepare drugs in MGIT 960 SIRE Kit	1. Reconstitute each **S**treptomycin lyophilized drug vial with **4 ml** of sterile distilled/deionized water to make a stock solution of 83µg/ml. 2. Reconstitute each **I**soniazid lyophilized drug vial with **4 ml** of sterile distilled/deionized water to make a stock solution of 8.3µg/ml. 3. Reconstitute each **R**ifampicin lyophilized drug vial with **4 ml** of sterile distilled/deionized water to make a stock solution of 83µg/ml. 4. Reconstitute each **E**thambutol lyophilized drug vial with **4 ml** of sterile distilled/deionized water to make a stock solution of 415µg/ml.
Prepare drugs in MGIT 960 PZA Kit	1. Reconstitute PZA drug vial with **2.5 ml** of sterile distilled/deionized water to make a stock solution of 8000 µg/ml.

Store lyophilized drugs at 2-8ºC upon receipt and reconstitute prior to use. Once reconstituted, aliquot any leftover drug solutions and freeze at -70 to -80ºC (±10°C) up to 6 months or up to the date of original expiry, whichever comes first. Once thawed, discard any leftover drug and do not store or refreeze.

12.2.2 Second Line Drug Stock Preparation

Source of drugs

All second-line drugs will be obtained in chemically pure form from Sigma or the appropriate pharmaceutical company. Acceptable drug powders are labeled with the generic name, its assay potency (usually expressed in micrograms [µg] of drug per mg of powder), and its expiration date. The powders are stored as recommended by the manufacturer. When removed from the freezer, it must come to room temperature before it is opened to avoid condensation of water.

Check the "Certificate of Analysis" (COA) provided by Sigma for each drug/lot number. If the COA is not included with the antibiotic shipment, it can be downloaded by lot number from the Sigma web site. Contact the supplier or manufacturer if any value is missing or cannot be clearly determined from the COA. If potency information is not included on the COA, it may be determined from the formula below.

Weighing drugs

All antimicrobial agents are assayed for standard units of activity. The assay units may differ widely from the actual weight of the powder and often may differ between drug production lots. Thus, the lab must standardize the antimicrobial solutions based on potency of the individual lot of each drug powder.

Formula for potency of drug

Potency = (assay purity) x (active fraction) x (1 – water content)

In some cases the potency may be expressed as a percentage. The following example shows how to calculate the potency in units of **µg/mg (w/w)**:

- Assay purity = 99.8%
- Measured water content =12.1%
- Active fraction = 100%

Calculation:

Potency = (assay purity) x (active fraction) x (1 – water content)

Potency = (998) x (1.0) x (1– 0.121) = **877 µg/mg or 87.7%**

The antimicrobial powder must be weighed on an analytical balance that has been calibrated with standard weights. If possible, weigh more than 100 mg of powder. To calculate the appropriate amount of solvent needed to obtain this concentration, the potency of the drug powder must be considered.

It is advisable to weigh out a larger amount of the drug than required (for the specific concentration) and then calculate the volume of solvent needed to obtain the required concentration, using the formula below.

Solvent for drugs

Follow the manufacturer's guidelines on the COA for recommendation of solvent. If a solvent other than water is recommended, only use sufficient solvent to solubilize the antimicrobial powder, and then dilute to the final stock concentration with sterile distilled water. For example, if the recommended solvent for Ofloxacin is 0.1 N NaOH:

1. Add weighed drug to a sterile tube, e.g., 50 ml Falcon tube.
2. Prepare 0.1 N (0.1 M) NaOH. For example, dissolve 4 g NaOH in 1 liter sterile distilled water.
3. Add 0.1 N NaOH solution drop-wise, shaking gently after each addition. Add NaOH just until drug dissolves completely and the solution is clear.
4. Finish diluting drug up to the appropriate volume (calculated below) with sterile distilled water.

Formula for volume of solvent

$$Volume = \frac{(Actual\ weight) \times (Potency)}{(Desired\ concentration)}$$

Example: To prepare a stock solution containing 10,000 µg/ml of kanamycin with powder that has a potency of 750 µg/mg, 170 to 200 mg of powder should be accurately weighed. If the actual weight is 180.0 mg, the volume of solvent needed is as follows:

$$Volume = \frac{180.0\ mg \times 750\ \mu g/mg}{10{,}000\ \mu g/ml} = 13.5\ ml$$

All stock solutions must be made at least 1,000 µg/ml, or ten-fold higher than the drug's working concentration (see table below), and sterilized by membrane filtration (0.22 µm pore size), unless otherwise recommended by the manufacturer.

Dispense small volumes of sterile stock solutions into sterile cryovials, carefully seal, and store for up to 12 months at -70 to -80°C (±10°C), or up to the date of the original drug powder expiry, whichever comes first.

Preparing working solutions

Before inoculating MGIT tubes:

1. Thaw individual cryovials to room temperature.
2. Dilute as appropriate in sterile distilled water to achieve the correct working concentration and use without delay. Test concentrations for each drug and the volumes added to MGIT tubes are listed in the table below.
3. Discard excess drug and never re-freeze.

Table 12.1 Drug Concentrations for DST in MGIT

Drug	Concentration of drug in working solution (µg/ml)	Volume added to MGIT tubes for test (µl)	Final/Critical concentration in MGIT tubes (µg/ml)
MGIT STR	83	100	1.0
MGIT INH	8.3	100	0.1
MGIT RIF	83	100	1.0
MGIT EMB	415	100	5.0
MGIT PZA	8000	100	100
Amikacin	83	100	1.0[1]
Capreomycin	208	100	2.5[1]
Gatifloxacin[2]	–	–	–
Kanamycin[2]	–	–	–
Levofloxacin	166	100	2.0[1]
Moxifloxacin	20.75	100	0.25[1]
Ofloxacin	166	100	2.0[1]
Enviomycin[2]	–	–	–

1 World Health Organization. Policy Guidance on Drug Susceptibility Testing (DST) of second line anti-tuberculosis drugs. Geneva, WHO, 2008 (WHO/HTM/TB/2008.392).
2 WHO has not recommended a critical concentration for the testing of this drug in the MGIT 960 instrument. Laboratories testing this drug must submit a written rationale for the critical concentration used for testing, as well as any validation testing performed.

12.3 Preparation of Tubes for Susceptibility Testing

12.3.1 Preparation of SIRE

1. Label five 7 mL MGIT tubes for each test isolate with a study label that includes identifying information described in **Section** 6.2: Login of Sputum Specimens. In addition, label tubes with one of each of the following: GC (Growth Control), STR (streptomycin), INH (isoniazid), RIF (rifampicin), EMB (ethambutol).

2. Place the tubes in the following sequence in the 5 tube AST set carrier, from left to right: GC, STR, INH, RIF, EMB.

3. Aseptically add 0.8 mL of BACTEC MGIT SIRE Supplement (provided in the SIRE kit) to each SIRE tube. It is important to use the supplement supplied with the kit.

4. Aseptically pipette 100 µl of the appropriately reconstituted drug into the corresponding MGIT tube; e.g., add 100 µl of the 83 µg/mL MGIT STR solution to the MGIT tube labeled "STR", etc.

5. It is important to add the correct drug to the corresponding tube.

6. Do not add drugs to the MGIT GC tube.

12.3.2 Preparation of PZA

When preparing PZA tubes, it is important to use the PZA media tubes and 'PZA Supplement' supplied with the PZA kit. Do not use the conventional MGIT tubes or the 'SIRE supplement', as the pH is different for both PZA medium and supplement.

1. Label two 7mL MGIT PZA media tubes for each test isolate with a study label that includes identifying information described in Section 6.2: Login of Sputum Specimens. In addition, label tubes with one of each of the following: GC (Growth Control) or PZA (pyrazinamide).

2. Place the tubes in the following sequence in the 2 tube AST set carrier, from left to right: GC, PZA.

3. Aseptically add 0.8mL of BACTEC MGIT PZA supplement to each PZA tube.

4. Aseptically pipette 100uL of the 8000µg/ml MGIT PZA solution to the appropriately labeled MGIT tube.

5. Do not add drugs to the MGIT GC tube.

12.3.3 Preparation of Second-Line Drugs

NOTES:

- If second-line drugs are entered into the MGIT instrument using one of the instrument's three possible "undefined drug" set configurations, a separate drug-free GC tube must be prepared for use in a separate carrier from the SIRE tubes.
- Alternatively, second-line drugs can be added behind a set of SIRE drugs using the 8-tube AST carrier, thus requiring only one GC tube.
- If needed, blank MGIT tubes (non-inoculated, drug-free) can be used to fill empty slots in a carrier.

1. Label 7 mL MGIT tubes for each test isolate with a study label that includes identifying information described in **Section** 6.2: Login of Sputum Specimens.

2. In addition, label tubes with the appropriate second-line drug name or abbreviation; e.g., AMK (amikacin), CAP (capreomycin), OFL (ofloxacin), etc.

3. If a separate control will be used, label a GC tube (Growth Control).

4. Load tubes in the carrier set in the same order consistently, ensuring that the GC tube is always in the left-most position.

5. Aseptically add 0.8 mL of BACTEC MGIT SIRE Supplement, or appropriate OADC Enrichment, to each MGIT tube. **Do not use MGIT 960 growth supplement or PZA supplement.**

6. Aseptically pipette 100 µl of the appropriately diluted drug into the corresponding MGIT tube. Refer to the table above for appropriate volumes and working solution concentrations of the second-line drugs.

7. It is important to add the correct drug to the corresponding tube.

8. Do not add drugs to the MGIT GC tube.

12.4 Inoculum for MGIT DST

Carry out all work with positive MGIT tubes in biosafety cabinet, using full PPE.

Bacterial density of the inoculum is critical to the correct performance of the susceptibility test; thus, the following instructions must be adhered to strictly. Cultures must not be used to set up DST if more than 5 days have elapsed after signaling positive.

12.4.1 Using an Inoculum from Positive MGIT Culture

1. Positive MGIT cultures must have pure growth of *M. tuberculosis* (ZN positive, BAP negative, MPT/MTB 64 antigen test positive; **Section** 10: Liquid Culture – Mycobacteria Growth Indicator Tube (MGIT) and **Section** 13: Rapid Identification of *M. tuberculosis* Complex Using an Immunochromatographic Assay) in order to be tested for drug susceptibility.

2. DST must not be set up on the same day a MGIT tube signals positive.

3. If the culture is worked up one or two days after signaling positive, it can be used directly to inoculate the DST MGIT tubes.

4. If the culture is used to set up DST between three and five days after signaling positive, dilute 1 ml of MGIT broth in 4 ml of sterile saline (1:5 dilution).

NOTE: If a laboratory routinely experiences x200 errors when performing DST in the local patient population, the 3-5 day culture can be used **undiluted** as a first step, as described in Flow Chart 4. Monitor this technique very closely to ensure that an excess of x400 errors is not subsequently produced.

5. If the culture has been positive longer than five days, subculture into a fresh MGIT tube:

    - Vortex MGIT broth well to mix thoroughly. Leave 5-10 minutes to allow any large clumps to settle.
    - Supplement a new MGIT tube with 0.8 ml Growth Supplement *without* PANTA.
    - Remove inoculum from the supernatant broth and make a 1:100 dilution of the positive MGIT tube into sterile saline or 7H9 broth.
    - Mix tube well by inverting gently several times.
    - Inoculate new MGIT tube with 0.5 ml of the 1:100 diluted specimen.
    - Cap tube tightly and mix well by inverting gently several times.
    - Enter tube into MGIT 960 instrument and monitor until it becomes positive.
    - Use new tube for DST tests from one to five days of positivity as described above.

12.4.2 Using an Inoculum from Positive LJ Culture

Carry out all work with positive LJ slants in biosafety cabinet, using full PPE.

1. DST can be performed with growth from positive LJ slants if the MGIT tube is contaminated or fails to grow *M. tuberculosis*. The culture must be identified as a pure growth of *M. tuberculosis* (ZN positive, MPT/MTB 64 antigen or molecular test positive; **Section** 11: Solid Culture: Lowenstein Jensen (LJ) Media and **Section** 13: Rapid Identification of *M. tuberculosis* Complex Using a Chromatographic Immunoassay).
2. Add 4 ml of Middlebrook 7H9 Broth (or BBL MGIT broth) to a 16.5 x 128 mm sterile tube with cap containing 6 – 10, 2 mm, glass beads (or use tube the same size as lab's McFarland standards).
3. Using a sterile loop or applicator stick, scrape as many colonies as possible from growth no more than 14 days old. Do not remove any solid medium. Suspend the colonies in the Middlebrook 7H9 broth.
4. Vortex the suspension for 2-3 min to break up the larger clumps. The suspension should exceed a 1.0 McFarland standard in turbidity.
5. Let the suspension stand for 30 min without disturbing. Sediment should settle to the bottom of the tube.
6. Transfer the supernatant fluid to another 16.5 x 128 mm sterile tube with cap (avoid transferring any of the sediment) and let the suspension stand for another 15 min.
7. Transfer the supernatant fluid (it should be smooth, free of any clumps) to a third 16.5 x 128 mm sterile tube.
8. Using 7H9 broth, adjust the suspension to a 0.5 McFarland standard. Do not adjust below or above a 0.5 McFarland Standard.
9. Dilute 1 ml of the adjusted suspension in 4 ml of sterile saline (1:5 dilution) using a new 16.5 x 128 mm sterile tube. This diluted inoculum is used for preparation of the MGIT DST tubes.

12.5 Growth Control Tube Preparation and Inoculation

12.5.1 For preparation of SIRE and second-line Drug GC Tube(s)

1. Vortex original MGIT tube, diluted MGIT culture, or diluted inoculum from LJ and mix well; let suspension settle for 5-10 minutes.
2. Aseptically pipette 0.1 ml of the organism suspension into 10 ml of sterile saline to prepare the 1:100 GC suspension (1% growth control).
3. Mix the GC suspension thoroughly by gently inverting 3-4 times.
4. Inoculate 0.5 ml of the 1:100 GC suspension into all MGIT tubes labeled "GC" for that specimen, using a micropipettor and sterile aerosol resistant tips.

12.5.2 For preparation of PZA GC Tube

1. Vortex original MGIT tube, diluted MGIT culture, or diluted inoculum from LJ as applicable, to mix well; let suspension settle for 5-10 minutes.
2. Aseptically pipette 0.5 ml of the organism suspension into 4.5 ml of sterile saline to prepare the **1:10** Growth Control suspension.
3. Mix the Growth Control suspension thoroughly by gently inverting 3-4 times.
4. Inoculate 0.5 ml of the **1:10** Growth Control suspension into the **PZA MGIT** tube labeled "GC" for that specimen, using a micropipettor and sterile aerosol resistant tips.

NOTE: It is important to use an appropriately prepared **1:10** dilution for the "GC" tube for PZA DST to ensure accurate results and avoid PZA AST set errors.

12.6 Inoculation of Tubes Containing Test Drugs

1. Vortex original MGIT tube, diluted MGIT culture, or diluted inoculum from LJ as applicable, to mix well; let suspension settle for 5-10 minutes.
2. Aseptically pipette 0.5 ml of the organism suspension from the supernatant into all drug-containing tubes (STR, INH, RIF, EMB, PZA, any 2nd line drug tubes), using a micropipettor and sterile aerosol resistant tips – a separate tip must be used for each tube. Take care not to disturb the sediment.
3. Tightly recap the tubes. Mix tubes by gently inverting 3-4 times.

12.7 Entering and Removing AST Sets in the MGIT 960 Instrument for SIRE and PZA

Place the tubes in the appropriate carrier set, ensuring they are in the correct sequence from left to right:

- For SIRE, use the 5 tube AST carrier: GC, STR, INH, RIF, EMB (or the 8 tube carrier, if applicable)
- For PZA, use the 2 tube AST carrier: GC, PZA

Open desired drawer in MGIT instrument, and follow instructions below.

Function	Directions
Entering new AST sets	1. Press the <**tube entry**> soft key. 2. Scan the AST carrier's barcode label. The carrier barcode indicates an AST set, how many tubes are in the set, and the set's sequence number. For PZA testing, select PZA as the drug in the two tube carrier definition as it is a longer protocol. 3. Scan the accession barcode, if available; if not present, press the <**accession barcode not available**> soft key. 4. The display shows the default carrier set. Check that the AST set definition is displayed correctly for the AST set currently being entered. 5. The station LEDs of all the assigned stations for the set illuminate GREEN. Insert the tube set into the indicated stations, ensuring that the Growth Control tube is located in the leftmost indicated station. Make sure that all the tubes AND the carriers are fully seated in the drawer. 6. Repeat Steps 1 – 5 for each of the AST sets you want to enter. 7. When finished, close the drawer and wait a moment while the drawer performs a "quick scan" of drawer contents. If you have misplaced any AST sets, the quick scan can detect this and put the affected set into error status. Correct any errors before the MGIT 960 begins testing.
Removing completed AST sets (indicated by a red "+" on the drawer)	1. Open the desired drawer. Press the <**remove completed AST sets**> soft key. 2. The first completed AST set stations illuminate with FLASHING GREEN indicators. 3. Remove the carrier, starting with the completed set closest to the front of the drawer, and scan its barcode label. The LEDs at this station extinguish. 4. Repeat steps 2 – 3 to remove additional AST sets. 5. Place completed AST sets in the AST tube rack.
Printing an "Unloaded AST Set" report	1. Press the <**printer**> soft key to access report selection. 2. Press <**unloaded AST sets report**> soft key. 3. Match the AST sets with the printed report, and resolve any discrepancies.

NOTE: If a station error has occurred, the "!" indicator will illuminate YELLOW. Refer to the BACTEC MGIT 960 System's AST manual, Chapters 4 and 6, for assistance in resolving this error.

12.8 Interpretation of DST Results for SIRE and PZA

The BACTEC MGIT 960 instrument continually monitors all tubes for increased fluorescence. Analysis of fluorescence in the drug-containing tubes compared to the fluorescence in the Growth Control tube is used to determine susceptibility results. When the growth unit (GU) of the growth control reaches 400 within 4-13 days (SIRE) or 4-21 days (PZA), the GU values of the drug-containing vials are evaluated.

- S = **Susceptible** = the GU of the drug tube is less than 100
- R = **Resistant** = the GU of the drug tube is 100 or more

Invalid tests are reported when certain conditions occur that may affect test results:

- X ### = Error or Indeterminate results; reported when certain conditions occur that may affect the test. If possible, determine the cause of error. In any case, repeat testing with a pure culture of the isolate.
  - X200 = System cannot detect sufficient indication of growth in the Growth Control tube in the specified protocol time, and does not provide an interpretation of the AST set results. Often a result of too little inoculum, nonviable organisms, or a slow-growing drug-resistant strain. Please refer to Flow Chart 4 for further instructions.
  - X400 = System detects indications of possible contaminated or overinoculated tube, and does not provide an interpretation of the AST set results. Check the tube for turbidity and subculture to a blood agar plate to rule out contamination of the specimen. Please refer to Flow Chart 5 for further instructions.
  - Other conditions, such as power failure.

NOTES:

1. Observe all 'resistant' tubes visually for evidence of contamination when first removed from the instrument. Perform a ZN stain on any suspicious tube and subculture to a BAP. In addition, when drug resistance is observed and the patient's isolate has not been tested before, or if the isolate was not previously resistant to the drug, test tube(s) with ZN and BAP to ensure that growth is not due to contaminants or MOTT.

2. DST for SIRE and PZA should only be repeated once if the first test fails. If a valid result cannot be obtained after the second attempt, the test should be reported as 'TF' (test failed) and a Note to File should be written to document the DST failure.

3. If DST results for isoniazid or rifampicin are inconsistent with previous results for the same patient, review the results and QC and repeat the test. If the repeat result is discrepant with the first result, repeat the test a third time and record the third test result as the tiebreaker.

12.9 Entering and Removing AST Sets for Second- line Drugs

There is no means for designating the drug name or concentrations of second-line drugs in the MGIT system. Therefore, a site-specific procedure must be developed for documenting second line drug testing in MGIT. There are two options to perform second-line DST:

1. Manual interpretation – Use AST set carrier and enter in the instrument as "Undefined" drugs. In this case, interpretation of results is done manually using the GU values from the unloaded AST set report.

2. Instrument interpretation – Use AST set carrier and enter in the instrument as a first-line drug protocol. Each second-line drug is coded with the name of one of the first-line drugs. The instrument will interpret results automatically when the test is complete.

For both manual and instrument interpretation, the following procedures for correcting the MGIT unloaded AST reports must be written and performed by all applicable staff:

- Instructions for preparing the antibiotic stock solutions and dilutions needed to achieve the working solutions
- AST carrier(s) used
- Configuration of the drugs in the AST carrier set(s)
- Approved abbreviations for the full drug names
- Process for clarifying the drug names on the MGIT printout
  - Cross out each "undefined" or first-line drug name using one line through the printed drug name, and add the applicable 2nd line drug name, or approved abbreviation, next to it.
  - Cross out each concentration listed for the first-line drug, and replace it with the appropriate concentration for the actual drug tested. If "undefined" protocol is used, simply write in the concentration tested for each drug.
  - Initial and date the corrected MGIT report after all drug names and concentrations have been updated

Entering and removing AST carrier sets is the same as described above in Section 12.7, with one extra step of changing the AST set definitions (if applicable). Open desired drawer in MGIT instrument, and follow instructions below.

Function	Directions
Entering new 2nd-line AST sets	1. Follow steps 1-4 in "Entering New AST sets" above. 2. Press the <**change AST set definition**> soft key. 3. Use the UP ARROW or DOWN ARROW key to scroll through the available definitions for the scanned carrier size (As you scroll, the default set is indicated by a check mark in the main body of the display). 4. Press the <**okay**> soft key to select the highlighted set. The number of "undefined drugs" must correlate with the number of second-line drugs being tested. 5. Follow steps 5-7 in "Entering New AST sets" above.
Removing completed AST sets (indicated by a red "+" on the drawer)	1. Open the desired drawer. Press the <**remove completed AST sets**> soft key. 2. The first completed AST set stations illuminate with FLASHING GREEN indicators. 3. Remove the carrier, starting with the completed set closest to the front of the drawer, and scan its barcode label. The LEDs at this station extinguish. 4. Repeat steps 2 – 3 to remove additional AST sets. 5. Place completed AST sets in the AST tube rack.
Printing an "Unloaded AST Set" report	1. Press the <**printer**> soft key to access report selection. 2. Press <**unloaded AST sets report**> soft key. 3. Match the AST sets with the printed report, and resolve any discrepancies.

NOTES

- Remember to load tubes into the carrier set each time in the same order, i.e., with the same drug sequence (growth control, undefined drug #1, undefined drug #2, etc.).
- For further information, please see instructions in the BD MGIT DST manual; appendix A of the manual lists all possible AST set configurations.

12.10 Interpretation of DST Results for Second-Line Drugs

For all set configurations except the "undefined drug" protocol, when the growth unit (GU) of the GC tube reaches ≥400 within the timed protocol, the instrument marks the AST set complete and interprets the results. For undefined drugs only, GUs are recorded on the unloaded set report; however, susceptibility is not interpreted by the instrument. The interpretation must be made manually for the "undefined" option according to the following criteria:

- S = **Susceptible** = the GU of the drug tube is less than 100
- R = **Resistant** = the GU of the drug tube is 100 or more

NOTES:

1. Errors, e.g., x200 and x400, are generated the same as for first-line drugs depending upon the AST set configuration used for testing, and necessitate repeating as described in **Section** 12.9 above.
2. DST for second line drugs should only be repeated once if the first test fails. If a valid result cannot be obtained after the second attempt, report the test as "TF", provide a brief comment in the DST "Comments" section of Appendix B, and write a Note to File to document the DST failures.
3. If DST results for any of the fluoroquinolones or injectable agents are inconsistent with previous results for the same patient, review the results and QC and repeat the test. If the repeat result is discrepant with the first result, repeat the test a third time and record the third test result as the tiebreaker.

12.11 DST for Enviomycin, Gatifloxacin, and Kanamycin

WHO does not currently recommend critical concentrations for Gatifloxacin, Kanamycin, or Enviomycin in the MGIT system. However, some labs are testing these drugs as part of standard of care.

DST results will be accepted for:

- Gatifloxacin and/or Kanamycin in MGIT or solid media, so long as the laboratory submits a written rationale for the critical concentration used for the drug, as well as any validation testing performed.
- Enviomycin on Ogawa media at the critical concentration of 20 µg/ml.

The lab should follow their internal solid medium DST procedures, any applicable manufacturer's recommendations, as well as any appropriate quality control and assurance activities described in **Section** 16: Quality Assurance. All DST reporting guidelines outlined below are applicable to DST on solid media.

12.12 Reporting of DST Results for All Drugs

Record "**Susceptible**", "**Resistant**", or "**Test Failed**" on the internal lab worksheet/book and the lab requisition form (if applicable). Also, keep the MGIT printouts for all DST results with the patient records.

12.13 Internal Quality Control

It is extremely important to perform quality control on the drug sensitivity testing procedure. This testing must be performed:

- For each new batch of reagents (MGIT drug kits, other drugs, media, etc.)
- Weekly, in a DST run when patient tests are run weekly
- With each batch of patient isolates, when DST is performed less frequently.
- Using a pan-susceptible strain, such as H37Rv, which is sensitive to all of the test drugs

Record these results on Appendix F - DST QC Form. See **Section** 16: Quality Assurance.

NOTE: If the laboratory chooses to include other strains (such as clinical isolates with resistance to one or more of the second-line drugs) for quality control of drug susceptibility testing, either upon receipt of new drug lots, or at regular intervals, these results should be recorded on Appendix F - DST QC form.

12.14 Study Data Reporting

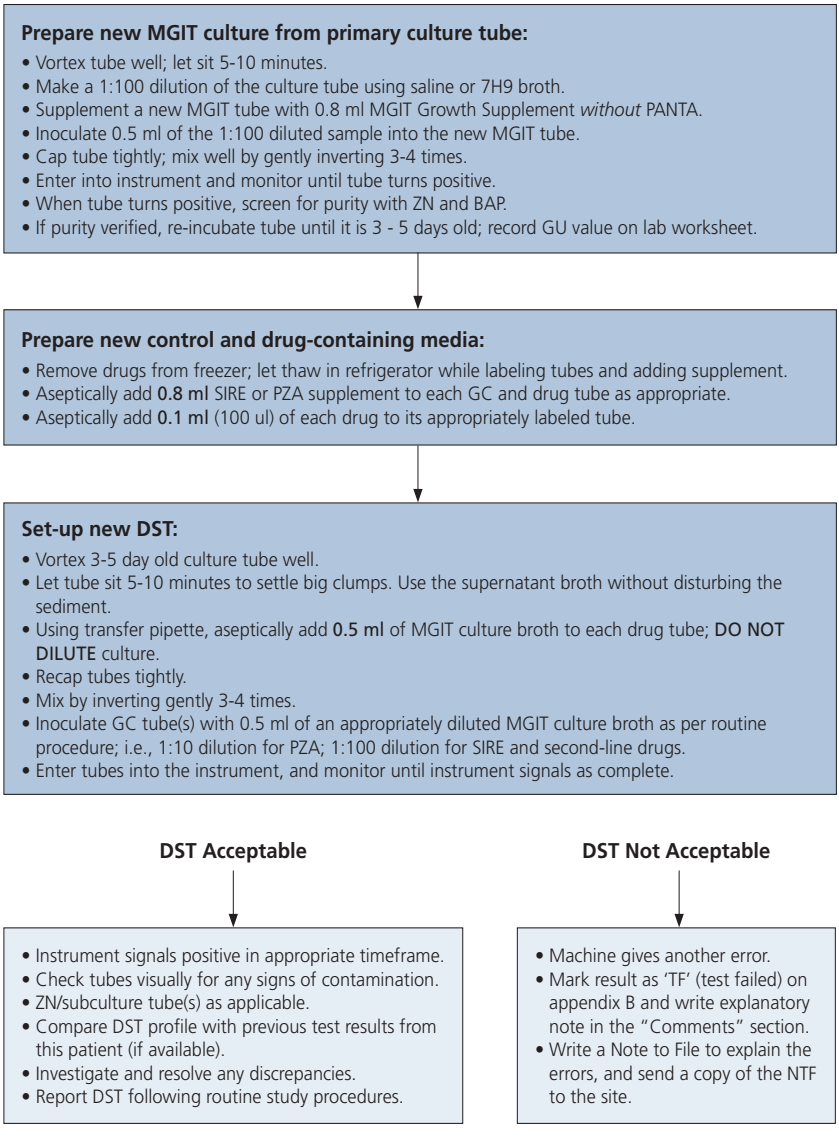
1. If a specimen was not positive for MTB or the visit interval did not require DST testing, mark "Not Applicable or Not Required by Protocol" on Appendix B.
2. Report susceptibility testing results on Appendix B for the specimen tested as follows:
   - Mark each drug result as "Susceptible (**S**)", "Resistant (**R**)", "Test Failed (**TF**)" or "Not Tested (**ND**)"
   - Record the testing method, date result was obtained, and the critical concentration of each drug tested.
   - If any discrepant results are seen, or retesting is performed, note this occurrence in the DST "Comments" section.
3. If the other specimen (#1 or #2) for the same visit was also positive for MTB, mark the non-tested specimen as "Not Applicable or Not Required by Protocol" on the associated Appendix B.
4. For INH, RIF, any fluoroquinolone or any injectable drug only, if a subsequent test result is inconsistent with previous results for a patient (especially if the drug profile changes from

"R" to "S"), review data and repeat the test. If the repeat result is discordant with the 1st test result for the current visit, repeat the test a 3rd time and record this as the final result on Appendix B.

5. If a specimen was positive for MTB complex at a time point requiring DST, but the isolate was unavailable for testing, e.g., culture contaminated and could not be purified, report susceptibility results for the individual drugs as "ND" and add an explanation in the DST "Comments" section.

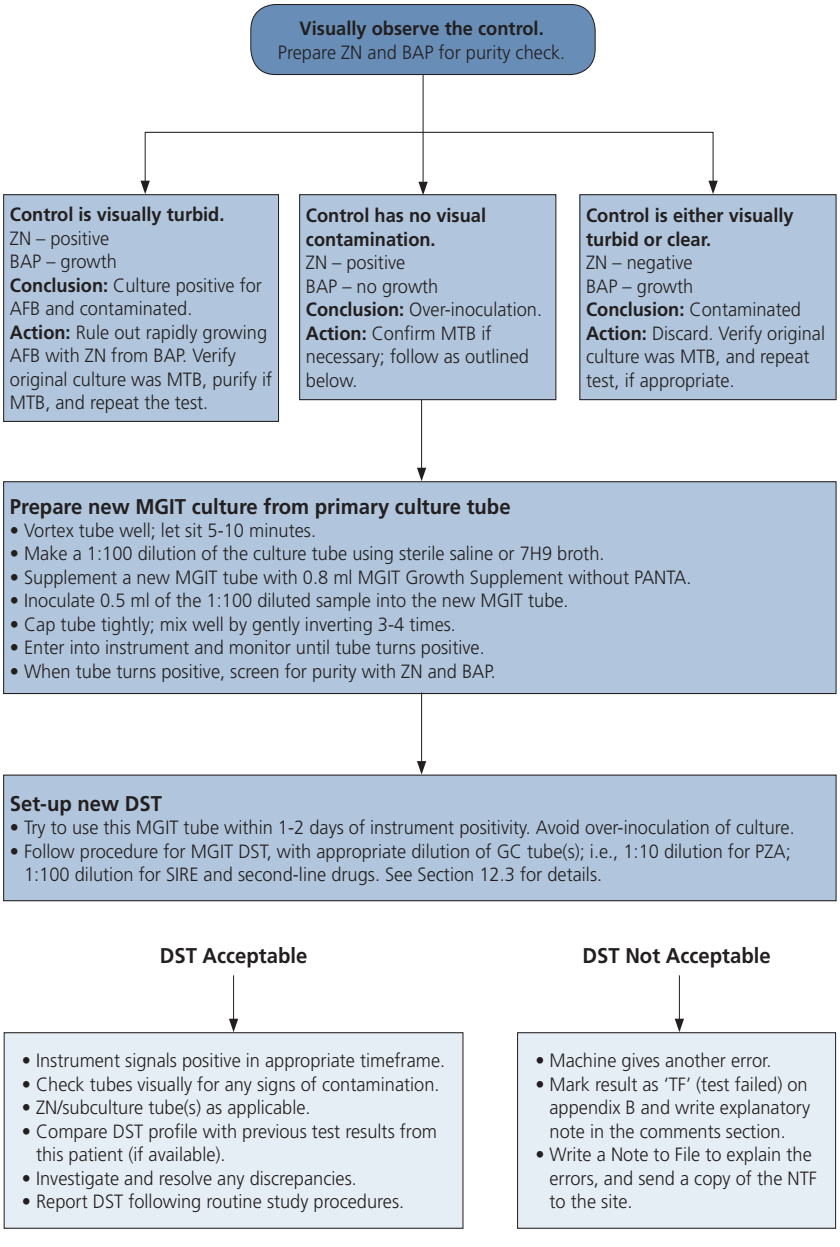
Flow Chart 4 Invalid x200 Errors from MGIT DST

NOTE: All work must be done in the biological safety cabinet.



Flow Chart 5 Invalid x400 Errors from MGIT DST

NOTE: All work must be done in the biological safety cabinet.



13 RAPID IDENTIFICATION OF M. TUBERCULOSIS COMPLEX USING AN IMMUNOCHROMATOGRAPHIC ASSAY

Purpose

To rapidly (< 1 h) and accurately detect *Mycobacterium tuberculosis* complex (MTB) in MGIT and LJ AFB-positive cultures without special instruments or equipment.

To differentiate MTB from mycobacteria other than tuberculosis (MOTT) for the effective treatment of the disease. To ensure consistency across all participating sites, only the Becton Dickinson (BD) MGIT TBc Identification Test (TBc ID) or the Tauns Capilia TB-Neo Test (Capilia) will be used. Definitive identification will be performed at every timepoint from all AFB-positive MGIT and LJ cultures.

Principle

A rapid immunochromatographic assay will be used to differentiate MTB and MOTT. BD's MGIT TBc ID and Tauns' Capilia TB are both lateral flow immunochromatographic assays. The BD assay detects MPT64 antigen, while Capilia detects MPB64 antigen, a mycobacterial protein that is specifically secreted from MTB cells during culture. When a sample is added to the test device, MPT64/MPB64 antigen binds to anti-MPT64/MPB64 antibodies conjugated to colloidal gold particles present on the test strip, forming an antigen-antibody complex. This antigen-antibody complex then migrates across the test strip to the reaction area, where it is captured by a second specific MPT64/MPB64 antibody fixed to the membrane. If MPT64/MPB64 antigen is present in the sample, a color reaction is produced by the labeled colloidal gold particles and is visualized as a pink (or purple) to red line. An internal positive control is included to validate proper test performance. The test will detect the following species of MTB complex: *M. tuberculosis, M. bovis, M. africanum*, and *M. microti*.

These tests have been shown to be highly sensitive (> 95%) and specific (> 95%) in a number of studies conducted in clinical settings.

Procedure

Materials

- TBc ID Test device or Capilia TB Test device
- Capilia extraction buffer (commercially available) or in-house prepared TBc ID extraction buffer (KH_2PO_4 , NaCl , Tween 80)
- Clean cylinder and media flask or bottle
- Analytical balance
- Weigh boats
- Distilled water
- Vortex mixer
- Timer
- 200 µL micropipette
- 200 µL sterile aerosol resistant tips

- Sterile 2 mL cryovials
- 10 µL sterile disposable loops
- Waste receptacle with biohazard bag and tuberculocidal disinfectant
- Permanent marker

Forms

- Lab-specific forms for recording ID test results
- Appendix G - Identification QC Form or use site-specific form if available and equivalent)
- Appendix B– Study Source Document

13.1 Preparation of TBc ID Extraction Buffer

Buffer formula

- KH_2PO_4 - 0.01 M
- NaCl - 0.145 M
- Tween 80 - 0.01%

To prepare 1 L of buffer

1. Using weigh boat and analytical balance, weigh out 1.36 g of KH_2PO_4.
2. Weigh out 8.5 g of NaCl.
3. Dissolve powders in 500 mL distilled water in a volumetric flask.
4. Using a micropipette, add 100 µL of Tween 80; pipette up-and-down repeatedly to dislodge all Tween material.
5. Mix thoroughly and bring to a final volume of 1 L with distilled water, using a cylinder.
6. Aliquot buffer into smaller volumes, e.g., 250 ml, before autoclaving. Label containers with the buffer name, date prepared, expiry date, batch lot number, and initials of preparer.
7. Sterilize for 15 minutes at 121°C, 15psi.
8. Store buffer at 2-8°C for up to 6 months.
9. Before each use, check buffer visually for signs of contamination or degradation, and bring to room temperature.

13.2 Specimen Preparation

Specimen preparation and subsequent steps must be performed in a BSC, using full PPE.

13.2.1 From positive MGIT tubes:

1. Ideally, test AFB smear-positive MGIT tubes within 5 days of instrument positivity.
2. Vortex the tightly capped MGIT tube for 30 seconds to ensure the suspension is well- mixed.

13.2.2 From positive LJ slants:

1. Test 2-4 week old growth.
2. Add 200µL of TBc ID extraction buffer or Capilia extraction buffer to a sterile cryovial.
3. Using a sterile 10 µL loop, scrape a loopful of several colonies and mix with buffer, avoiding any solid medium and/or contaminants present.
4. Vortex the cryovial for 30 seconds to create a uniform suspension.
5. For TBc ID, be sure the suspension turbidity is adjusted to approximately 0.5 McFarland.
   a. Insufficient density in the suspension can lead to false negative results.
   b. Subculture to a new LJ slant to obtain adequate growth and repeat testing as necessary.

NOTES:

1. Positive MGIT tubes can be stored at 2-37°C and tested in the **TBc ID** for up to 10 days after positivity; testing can be performed for two additional months when tubes are stored at –20°C to 8°C.
2. Positive cultures in liquid media and colonies on solid media can be tested with Capilia up to one year when stored at –20°C or at 2 to 8°C.

13.3 Inoculation of ID Test Device

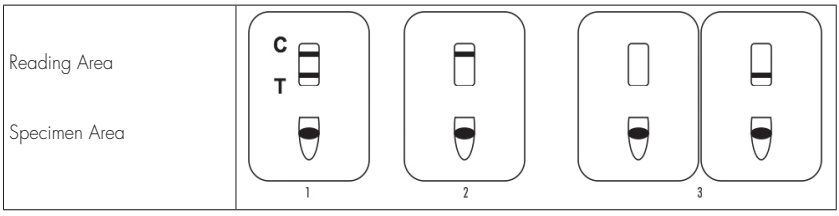
The rapid ID test device should be stored at 2-30°C (preferably refrigerated at 2-8 °C). Direct sunlight, excessive humidity, and high temperatures should be avoided. Foil pouches containing devices should not be opened until test is to be performed. Avoid touching the specimen well on the device with your hands.

1. If devices are refrigerated, bring to room temperature in the foil pouch prior to testing.
2. Place the device on a flat surface inside the BSC. Remove the rapid ID device from its foil pouch immediately before testing.
3. Label one device for each specimen to be tested with a study label containing the identifying information described in **Section** 6.2: Login of Sputum Specimens.
4. Place 100µL of specimen, either MGIT culture or bacterial suspension from LJ slant (see **Section** 13.2.2 above), into the specimen well of the test device. Change pipette tips between specimens.
5. Start timer for 15 minutes.
6. Examine the reading area of the test device after 15 minutes and record test results. **Do not interpret test after 60 minutes.**

13.4 Interpretation of Results

The following pictures are specific to the BD TBc ID kit. However, the TAUNS Capilia TB test is interpreted in the same manner.

Table 13 Interpretation of Results



1. **Positive:** Pink/purple to red lines form on the reading areas labeled Test [T] and Control [C] of the device.
2. **Negative**: A pink/purple to red line forms on the reading area labeled [C] of the device, but not [T].
3. **Invalid**: If no line is observed on the reading area labeled [C], technical errors or product damage has occurred. In this case, the test should be considered invalid and repeated using a new device.

NOTES

1. If the rapid ID test is negative, but the AFB smear and morphological characteristics of the isolate are consistent with MTB, re-incubate the MGIT tube at 37°C (±1°C) and repeat rapid ID test after 48 hours. If MTB is now detected, mark the result as "positive for MTB complex" on Appendix B. In addition, note in the MGIT "Comments" section that test was repeated.
2. It is preferable to test pure cultures without contamination, although observations show that slight contamination with bacteria does not interfere with the test.
   - For MGIT cultures, decontaminate the culture and repeat testing if necessary.
   - For LJ cultures, attempt to subculture a few colonies well-separated from the contaminants and repeat testing if necessary.

   However, grossly contaminated cultures may cause interference; interpret with caution.
3. *Staphylococcus aureus* is known to produce protein A, which may interfere and/or cause false positive results in all lateral flow assays.
4. A negative test does not always rule out MTB, as mutations are known to arise in the MPT/MPB 64 gene. If MTBc is highly suspected, and identification results persist in being negative, refer to Flowchart 2A for additional testing algorithms using a molecular assay (HAIN GenoType® Mycobacterium CM or GenoType® MTBDRplus).
5. If the test is invalid:
   - Investigate causes for the invalid result and try to resolve; e.g., decontaminating a heavily contaminated culture.
   - Repeat the test.
   - If test remains invalid, refer to Flowchart 2A for additional testing algorithms using a molecular assay (HAIN GenoType® Mycobacterium CM or GenoType® MTBDRplus).
6. Record observations for invalid or uninterpretable results; e.g., a contaminated culture.

13.5 Reporting of Identification Results

Record results, including all repeat tests; as "positive for MTB complex", "negative for MTB complex", or "invalid test" on the laboratory worksheet.

13.6 Internal Quality Control

A positive and a negative control must be tested with each new lot or new shipment of kits received and with each new batch of extraction buffer prepared. Similarly, these controls must be run weekly, or along with each batch of patient isolates, when tests are set up less frequently. Refer to **Section** 16: Quality Assurance for details.

13.7 Study Data Reporting

1. If identification is not performed because culture is negative, mark "N/A" in the appropriate section on Appendix B.

2. If the test confirms the presence of MTB complex:

    - Record the Identification of AFB result as "Positive for MTB complex" in the MGIT or LJ culture section on Appendix B.

    - Record the test method (Capilia or TBc ID) and any comments in the MGIT or LJ culture section on Appendix B.

3. If the rapid ID test is negative for MTB complex:

    - Proceed with instructions for molecular testing in Flowchart 2A.

        – If MTB is detected, Record the Identification of AFB result as "Positive for MTB complex" in the MGIT or LJ culture section on Appendix B. Specify the ID test used to confirm MTB (HAIN GenoType® Mycobacterium CM or GenoType® MTBDRplus).

        – If MTB is not detected, record the Identification of AFB result as "Negative for MTB complex" in the MGIT or LJ culture section on Appendix B. Specify the ID test used to rule out MTB (HAIN GenoType® Mycobacterium CM or GenoType® MTBDRplus).

4. Record any comments in the MGIT or LJ culture section on Appendix B.

14 LONG-TERM STORAGE OF MTB ISOLATES

Purpose

This section provides detailed instructions on the proper preparation and freezing of MTB isolates.

Principle

MTB isolates from all positive cultures (using the LJ subculture from a MGIT tube positive for MTB, or a subculture from the original LJ culture, if MGIT is unavailable) will be frozen in 7H9 broth plus glycerol to preserve them for any repeat or additional microbiology tests that may need to be performed (either in-house or at a central laboratory). It is recommended that at least four aliquots from baseline visits, and two aliquots from subsequent visits, be frozen for each MTB isolate.

Procedure

Materials

- Positive LJ slants from **Section** 11: Solid Culture: Lowenstein Jensen (LJ) Media
- 2 ml sterile cryotubes with screw top, externally threaded
- Water bath
- 7H9 media with glycerol
- ADC or OADC enrichment broth
- Sterile transfer pipettes with graduations marking volume (individually wrapped)
- Sterile loop, disposable applicator stick, or cotton swab
- Permanent marker
- Cryobox and rack
- Study-specific labels

Forms

- Appendix B – Study Source Document Worksheet
- Appendix E – New Reagent/Media QC
- Appendix J – Freezer Storage Log for MTB Isolates

14.1 Preparation of Media

Note: The following instructions are for preparing media using Difco dehydrated base. Follow specific instructions if another manufacturer's base medium is used and adjust quantities if a smaller batch is needed

1. Suspend 4.7 g 7H9 Broth Base in 900 ml distilled water. Mix thoroughly.
2. Add 2 ml of glycerol; rinse pipette a few times with broth to dislodge all material.
3. Heat gently if necessary to dissolve the medium completely.
4. Autoclave at 121°C for 10 minutes. Cool to 45°C in a water bath.

5. Aseptically add 100 ml of ADC or OADC enrichment. Mix gently, but thoroughly.
6. Label container with media name, date prepared, expiry date, batch lot number, and initials.
7. Test media for sterility and performance. See Section16.2.1.2.2.
8. Store at 2-8°C up to 3 months; protect from any direct light.
9. Discard any prepared media that shows signs of contamination, discoloration, or evaporation.

14.2 Freezing MTB Isolates

Freezing of MTB Isolates must be performed in a BSC, using full PPE.

1. Label cryotube with study label containing screening and/or subject ID, visit number, aliquot or sputum specimen #, date cryotube is prepared, and lab accession number. See example below.

Screen ID: _____ Subj ID: _____

V#: _____ Aliq/Spec#: _____ Site#: _____

Date frozen: _____ Lab access #: _____

2. Dispense 1.0- 1.2 ml of 7H9 broth into cryotube using a sterile transfer pipette.
3. Check the label on the LJ tube and the cryotube to ensure they match before transferring growth from the slant to cryotube.
4. Using a sterile loop, disposable sterile applicator stick, or sterile cotton swab wetted with sterile saline or broth, transfer several sweeps of *M. tuberculosis* growth from LJ subculture (containing fresh, pure growth – **Section** 10.9: Subculture and Storage of MGIT Cultures) and emulsify in broth.
    - Use colonies showing good, confluent and pure growth within 10-15 days of first appearance. **Older cultures will not provide reliable, long term viability.**
    - Density of suspension should be greater than a 1.0 McFarland standard.
    - Do not scrape off any culture medium; this will give false turbidity.
5. Tighten cap and thoroughly mix suspension using a vortex.
6. Place cryotube in cryobox and freeze box in a rack or specified location in a -70 to -80°C (±10°C) freezer.

NOTES

1. If a frozen isolate is used in the lab for any reason (excluding shipping to central laboratory), a replacement cryotube must be prepared and frozen.
2. Freezer boxes must be organized to easily identify the isolates by patient and by sputum specimen number (#1 or #2), in order to retrieve these isolates for further testing or shipping.
3. Cryoboxes may contain isolates from more than one patient, but the second aliquot from each visit/sputum specimen (#1, #2) should be stored in a separate cryobox that exactly mirrors the first cryobox. Consider the following example:
    - Subj ID 1001
        - Visit 2 has a positive culture from one specimen. 
        - Visit 3 has a positive culture from one specimen. 

- Subj ID 1002
  - Visit 4 has positive cultures from two specimens - #1  and #2  .
  - Visit 5 has positive cultures from two specimens - #1  and #2  .

Cryobox 1

Subj ID 1001				Subj ID 1002			
V2 aliquot 1	V2 aliquot 2	V3 aliquot 1	V3 aliquot 2	V4 # 1	V4 # 2	V5 # 1	V5 # 2
							

Cryobox 2

Subj ID 1001				Subj ID 1002			
V2 aliquot 3	V2 aliquot 4	V3 aliquot 3	V3 aliquot 4	V4 # 1	V4 # 2	V5 # 1	V5 # 2
							

4. Record on the Freezer Storage Log for *M. tuberculosis* Isolates Form (Appendix J) the screening and/or subject ID, visit number, specimen # (if applicable), aliquot # (if applicable), total volume, date cryotube is prepared, and lab accession number. Also include the freezer number (if applicable), rack number, box number, and place/position numbers on the freezer storage log. If a rack is not used, record the location where the box is stored in the freezer.

5. Record on Appendix B that isolate was banked for long-term storage.

15 SHIPPING OF FROZEN ISOLATES

Purpose

This section provides instructions on the proper preparation and shipping of MTB isolates.

Materials

- Cryoboxes (plastic; size must be compatible with dimensions of lab-specific cryovials when labeled and wrapped with Parafilm)
- Tape
- Biohazard bags
- Absorbent material
- Dry Ice (to be provided by shipping vendor)
- Shipping container and boxes (to be provided by shipping vendor)
- Parafilm

Forms

- Shipping requisition form
- Shipping labels
- Airway bills
- Dangerous goods form
- Appendix B– Study Source Document Worksheet
- Appendix J– Freezer Storage Log for MTB Isolates

15.1 Isolate Shipping Schedule

When shipping isolates, it is recommended that duplicate tubes for each aliquot/specimen # be shipped in separate shipments, to prevent total loss of a particular visit isolate. An aliquot of each isolate shipped should be maintained at the lab.

15.2 Packaging of Isolates

NOTE: Isolates must be packaged by lab staff with certification in the packaging and shipping of Category A, infectious substances affecting humans, as defined by the International Air Transport Association (IATA).

1. Ensure that isolates have been frozen at -70 to -80°C (±10°C) for a minimum of 48 hours prior to shipping.
2. Wrap top of cryovials with Parafilm, ensuring they fit comfortably into the cryobox without damaging the orange label.
3. Follow specific instructions provided by the shipping vendor for shipping frozen isolates.
4. Fill out a separate shipping requisition form for each shipment of isolates.
5. File a copy of the requisition form(s) in the laboratory binder.

15.3 Study Data Reporting

Record on Appendix B whether or not an isolate was shipped.

1. If neither MGIT nor LJ culture was positive for MTB complex, mark "No".
2. If culture was positive for MTB complex, and isolate shipped, mark "Yes", and record aliquot number/isolate number and date of shipment for each as applicable.
3. If culture was positive for MTB complex, but isolate unavailable for shipping, e.g., culture contaminated and could not be purified, mark "No" and add explanation in the Shipping "Comments" section.

For all other visit intervals, which do not require an isolate to be shipped, mark "N/A" on Appendix B.

16 QUALITY ASSURANCE

Purpose

Quality assurance is a critical component of laboratory testing, as it ensures accuracy and consistency of laboratory test processes throughout the examination of a sputum specimen – from the point of collection to result reporting and database entry. In addition, good record keeping and periodic monitoring of the data generated will help guarantee that all lab procedures are performing properly.

Principle

The examination and monitoring of multiple parameters involved in testing are guided by standards mandated by accrediting agencies and the quality assurance guidelines in the Good Clinical Laboratory Practice (GCLP) standards. GCLP standards are recommended for international laboratories participating in clinical research, and help assure regulatory authorities that the data produced are a true reflection of the results obtained during the study. Quality assurance practices consist of:

Quality Control (QC)

- Internal QC – monitoring of test performance, media quality, reagent activity, DST accuracy, etc.
- External QC – proficiency testing to assess technical competency, test accuracy and reproducibility

Quality Monitoring (QM)

- Monitoring of critical practices, instrument function, data entry, contamination rates, and culture positivity rates that may affect the outcome of research results

Quality Improvement (QI)

- Continuous effort to improve service, function, workflow, customer satisfaction, etc.

All quality monitoring practices require documentation of results, corrective action and resolution of unacceptable results, along with supervisor review. Sample forms for documenting QC, QM, and QI procedures are included (Appendices C - N) or, if the laboratory has equivalent forms in use, these may be used for documentation after review and approval from the Sponsor. All QC results should be documented in the same manner as patient specimens, e.g., MGIT and LJ culture results, colony counts, etc.

16.1 Laboratory QC /QM Schedule

Table 16.1 Laboratory QC/QM Schedule

Daily or with each patient run	Weekly or with each patient run	Monthly	New lot # or batch #
Refrigerator, freezer, incubator, rooms, and centrifuge temperatures	Positive and negative controls for MGIT and LJ cultures	Internal quality assessment to improve microscopy results	Positive and negative controls for new staining reagents for AFB smears
Positive and negative controls for AFB smears	Reference strain for drug susceptibility testing	MGIT Time to detection QC for MTB reference strain	Sterility and performance testing of culture media – MGIT, LJ, 7H9 Broth, BAP
MGIT Maintenance (See **Section** 10: Liquid Culture – MGIT)	Positive, negative, and reagent controls for identification kits	Complete Monthly Data Monitors form	Reference strain testing for new lots of MGIT SIRE, PZA kits and other anti-TB drugs or drug kits
		MGIT QC Report (See **Section** 10: Liquid Culture – MGIT)	Positive, negative, and reagent controls for new lots of identification kits

16.2 Quality Control Activities

16.2.1 Media QC

16.2.1.1 McFarland Turbidity Standards

Many QC procedures involve preparing dilutions from a standardized suspension of organisms using a turbidity standard, so that the number of bacteria present will be within a given range.

According to CLSI guideline M24-A, each McFarland turbidity standard is equivalent to the turbidity of a bacterial concentration. For example, a McFarland No. 0.5 turbidity standard is equal to the turbidity of approximately 1.5 x 10^8 CFU/ml; a McFarland No 1.0 standard equals approximately 3 x10^8 CFU. The standard is compared visually or using a densitometer/spectrophotometer to a suspension of bacteria in sterile 7H9 broth and adjusted by adding more broth if too heavy, or more bacteria if too light. However, the latter must be done after making a single cell suspension, i.e., breaking up clumps with beads, allowing clumps to settle, and transferring the supernatant to a new tube. An inoculum that is too heavy or too light will adversely affect the results so it is important to adjust inoculums as closely as possible to the standard.

Preparation of Barium Sulfate Turbidity Standards

McFarland standards are commercially available and prepared from suspensions of latex particles, which lengthen the shelf life and stability of the standard. Alternatively, they can be made by mixing specified amounts of barium chloride and sulfuric acid. This forms a barium sulfate precipitate, which causes turbidity in the solution.

1. To prepare a McFarland No. 1 standard, mix 0.1 ml of 1.175% barium chloride dihydrate ($BaCl_2 \bullet 2H_2O$) with 9.9 ml of 1% sulfuric acid (H_2SO_4).
2. For a McFarland No. 0.5 standard, mix 0.05 ml of 1.175% barium chloride dihydrate ($BaCl_2 \bullet 2H_2O$) with 9.95 ml of 1% sulfuric acid (H_2SO_4).
3. Seal the capped tubes with wax, parafilm, or some other means of preventing evaporation.
4. Label the standards with the McFarland number, date of preparation, and date of expiration (6 months from preparation date).
5. Store standards in the dark at 20-25°C.
6. Discard after 6 months or sooner if any volume is lost.
7. Before each use, vortex standard vigorously, mixing the fine white precipitate of barium sulfate in the tube to achieve a uniform turbid appearance. Replace standard if large particles appear.

Ideally, the accuracy of the density of a prepared McFarland standard should be checked by using a densitometer or spectrophotometer with a 1 cm light path, assuring parameters correspond to the guidelines in the table below:

Table 16.2 Properties of McFarland Standards

McFarland Standard No.	0.5	1
1.0% Barium chloride	0.05 ml	0.1 ml
1.0% Sulfuric acid	9.95 ml	9.9 ml
% Transmittance*	74.3	55.6
Absorbance*	0.132	0.257

* at wavelength of 600 nm

Adjusting Turbidity for Inocula

1. Use tubes of the same size/diameter for preparing isolate suspensions and dilutions as the size of the McFarland standard tube.

2. If available, use a spectrophotometer or similar instrument to adjust the turbidities.

3. Use a "Wickerham" card as a visual guide for adjusting the turbidities when instrumentation is unavailable.

16.2.1.2 Laboratory-Prepared Media

Lab-prepared media must be thoroughly quality controlled. It is recommended to make small batches of media (e.g., every four weeks or more frequently) to ensure freshness and quality. 1-3% of each new batch of medium must be incubated to test for sterility and 1-3% must be tested for performance characteristics (ability to support a certain amount of growth in a specified time of incubation). Baseline criteria for optimal growth with standardized inocula and incubation periods should be established from results of several well-prepared batches.

The time to detection of growth, and number and size of colonies within a specified time of incubation should be critically evaluated. If a newly prepared batch of medium, when tested, yields results outside the established range, it must be considered unsatisfactory. Colony counts 20% above or below the established range may be considered unacceptable; however, it is preferable to establish lab-specific criteria (that is, determine mean CFU and standard deviation of several good prepared batches).

Required procedures for Sterility and Performance QC of each media type are detailed below. Media can be released for routine laboratory use after passing all QC checks for sterility and performance.

16.2.1.2.1 QC Protocol for LJ Medium

Sterility Check

1. *Frequency:* Each new batch of prepared medium.
2. *Controls:*
   a. 1-3% of LJ tubes from a batch (for example: for a batch of 100 tubes, select 2 tubes)
   b. Incubate for 14 days at 37°C (±1°C).
3. *Acceptable Results*: No growth on any tube. Visual inspection confirms proper color, texture, and homogeneity of medium.
4. *Corrective Actions:*
   a. If all tubes are contaminated, notify supervisor immediately, discard entire batch, and prepare new media.
   b. If one tube is contaminated, repeat exercise with at least 10 additional tubes.
   c. If >1 tube is contaminated upon repeat testing, notify supervisor immediately and discard entire batch.
   d. Investigate and resolve all problems, and then prepare new media.
5. *Documentation:* Record results on the Reagent/Media QC form – Appendix E. If contamination is seen, prepare an Appendix K form to document the corrective action.

Performance QC

1. *Frequency:* each new batch of prepared medium.
2. *Controls:* 1-3% of LJ tubes from a batch, tested with 10^{-2}, 10^{-3} and 10^{-4} dilutions of *M. tuberculosis* (H37Rv or H37Ra) in 7H9 broth (e.g., testing 2% of a batch of 100 tubes of media would include 6 tubes in total, 2 tubes inoculated with each of 3 working dilutions). See procedure below.
3. *Acceptable Results:* Growth on all tubes is consistent with *M. tuberculosis* and within 20% of the laboratory's own established reference ranges for each dilution.
4. *Corrective Actions:*
   a. If tubes show no growth, notify supervisor immediately, discard entire batch, and prepare new media.
   b. If growth still not in acceptable range after repeat testing, notify supervisor immediately and discard entire batch.
   c. Investigate and resolve problems, then prepare new media.
   d. If colony counts are lower than the acceptable range, check the preparation of the MTB suspension, especially if prepared from a frozen stock. Loss of viability is a consideration when freezing low concentrations of MTB.
5. *Documentation:* Record results on the Reagent/Media QC form – Appendix E. If QC results are not acceptable, prepare an Appendix K form to document the corrective action.

Preparation of MTB Culture Suspension and Working Dilution

1. Subculture a *M. tuberculosis* QC strain (H37Rv or H37Ra) onto several LJ slants.
2. Incubate the tubes at 37°C (±1°C), and observe growth visually.
3. Use colonies showing good, confluent, and pure growth within 10-15 days of first appearance. Old cultures do not give reliable results.
4. Remove growth from the slant by carefully scraping the colonies off the slant with a sterile loop or sterile wooden applicator stick. Take extreme precaution not to scrape off any culture medium (which gives false turbidity measurement).

5. Transfer growth into a screw cap tube containing 4 ml of sterile 7H9 broth and 6-10 glass beads (2 mm diameter), and vortex well.
6. Let tube sit for 30 minutes for clumped organisms to settle.
7. Transfer the supernatant fluid to another sterile tube (avoid transferring any of the sediment) and let the suspension stand for another 15 minutes.
8. Transfer the supernatant to a new tube and adjust turbidity of this suspension to a McFarland No.1 standard using 7H9 broth.
9. Prepare 3 serial 10-fold dilutions of the adjusted solution (10^{-2}, 10^{-3}, and 10^{-4}) in 7H9 broth.
10. Prepare 20-50 cryotubes for each dilution. Label with strain type, dilution, and expiration date (six months after preparation).
11. Aliquot 1.5 ml of each dilution to the appropriate cryotubes and freeze at –70°C (±10°C), up to six months.

Inoculation and Incubation

1. Remove one aliquot of each dilution (10^{-2}, 10^{-3} and 10^{-4}) from the freezer.
2. While thawing, bring LJ medium to room temperature.
3. With a micropipettor and sterile aerosol resistant tips (ART) tips, mix the working solution with the pipettor 2-3 times to ensure even distribution of MTB.
4. Inoculate LJ tubes with 200 µl of each dilution.
5. Incubate at 37°C (±1°C), reading weekly for 21-30 days.
6. Compare counts with the laboratory's established reference range.

16.2.1.2.2 QC for 7H9 Broth

Sterility Check

1. *Frequency:* Each new batch of prepared medium.
2. *Controls:* Number of tubes equivalent to 1-3% of total volume prepared.
3. *Acceptable Results:* No growth in any tube after 7 days of incubation.
4. *Corrective Actions:*
   a. If all tubes are contaminated, notify supervisor immediately, discard entire batch, and prepare new media.
   b. If one tube is contaminated, repeat exercise with at least 10 tubes.
   c. If >1 tube is contaminated upon repeat, notify supervisor immediately and discard entire batch.
   d. Investigate and resolve problems, then prepare new media.
5. *Documentation:* Record results on the Reagent/Media QC form – Appendix E. If contamination is seen, prepare an Appendix K form to document the corrective action.

Performance QC

1. *Frequency:* Each new batch of prepared medium.
2. *Controls:* Number of tubes equivalent to 1-3% of total volume prepared, tested with 10^{-2}, and 10^{-4} dilutions of *M. tuberculosis* H37Rv or H37Ra in 7H9 broth (e.g., 2% of a 100 ml batch of medium is 4 tubes in total, 2 tubes inoculated with each of two working dilutions). See procedure below.

3. *Acceptable Results:* Growth in all tubes is consistent with the appearance of *M. tuberculosis* (flocculent, granular) within 1-2 weeks.
4. *Corrective Actions:*
   a. If tubes do not show growth, notify supervisor immediately, discard entire batch, and prepare new media.
   b. If tubes show overall turbidity suggesting bacterial growth, confirm with ZN stain and/or subculture to solid media.
   c. If sterility check was acceptable and only inoculated tubes are contaminated, repeat performance QC with fresh culture of *M. tuberculosis*.
5. *Documentation:* Record results on the Reagent/Media QC form – Appendix E. If QC results are not acceptable, prepare an Appendix K form to document the corrective action.

Preparation of MTB Culture Suspension and Dilutions

Prepare dilutions as above in **Section** 16.2.1.2.1: QC Protocol for LJ Medium.

Inoculation and Incubation

1. Remove one aliquot of the 10^{-2} and 10^{-4} dilutions from the freezer.
2. While thawing dilutions, bring broth to room temperature. If not already aliquoted into tubes, aseptically dispense 4.5 ml 7H9 broth into appropriate number of sterile disposable tubes, e.g., 16.5 x 128 mm.
3. With a micropipettor and ART tips, mix the working solution with the pipettor 2-3 times to ensure even distribution of MTB, and inoculate tubes with 500 µl of each dilution. Vortex to mix.
4. Incubate tubes with caps loosened at 37°C (±1°C), reading weekly for 7-14 days.

Observe all tubes for evidence of growth, and note the approximate number of days for characteristic growth to appear.

16.2.1.2.3 QC Protocol for Blood Agar Medium

Sterility Check

1. *Frequency:* Each new batch of prepared medium.
2. *Controls:* 1-3% of total number of plates prepared, incubated at 35-37°C for 72 hrs.
3. *Acceptable results:* No growth on any plate.
4. *Corrective Actions:*
   a. If growth is seen on any plate, repeat sterility check using 10 additional plates.
   b. If >1 plate is contaminated upon repeat testing, notify supervisor immediately, and discard entire batch.
   c. Investigate and resolve problems, then prepare new media.

5. *Documentation:* Record results on the Reagent/Media QC Form – Appendix E. If contamination is seen, prepare an Appendix K form to document the corrective action.

Performance Check

1. *Frequency:* Each new batch of prepared medium.
2. *Controls:* 1-3% of total number of plates prepared, tested with diluted suspension of *E. coli or Staphylococcus aureus* (e.g., 2% of a 100 ml batch of medium is 4 tubes in total). See procedure below.

3. *Acceptable results:* Growth of typical colonies within 48 hours.
4. *Corrective Actions:*
   a. If no growth is seen, repeat performance check.
   b. If colonies are not typical of the bacteria used or the growth is mixed, repeat performance check with a fresh culture of the stock strain.
   c. If performance still not as expected upon repeat testing, notify supervisor immediately, and discard entire batch.
   d. Investigate and resolve problems, then prepare new media.

5. *Documentation:* Record results on the Reagent/Media QC Form – Appendix E. If QC results are not acceptable, prepare an Appendix K form to document the corrective action.

Preparation of Bacterial Suspension and Working Dilutions

1. The QC strain of *Escherichia coli or Staphylococcus aureus* can be a clinical isolate from the microbiology laboratory or reference strain.
2. To maintain a frozen stock of the control bacteria:
   a. Prepare a suspension from 24-hour growth from BAP in Tryptic Soy broth with 15% glycerol (or other appropriate storage medium) to the density of a McFarland No.1 standard.
   b. Aliquot ~1.5 ml volume into labeled cryovials.
   c. Freeze at -70 to -80°C (±10°C) for up to twelve months.
3. To prepare a fresh suspension: Twenty-four hours prior to use, streak one drop of thawed, well-mixed *E. coli or S. aureus* stock suspension onto a blood agar plate or other appropriate medium and incubate overnight at 35-37°C.

Inoculation and Incubation

1. Prepare a suspension of colonies from a solid bacteriologic medium in sterile distilled water to McFarland No. 0.5 standard. Alternatively, thaw the stock suspension and dilute to the same density.
2. Make a 100-fold dilution (10^{-2}) by adding a 10 µl suspension to 1 ml sterile distilled water. Mix well.
3. Make another 100-fold dilution, using 10 µl of the 10^{-2} dilution in 1 ml distilled water. Using this 10^{-4} dilution, inoculate the plate with10 µl and streak for isolation.
4. Incubate at 35-37°C for 48 hours, and check for growth with typical colony size and morphology.

16.2.1.3 Commercially-Prepared Media

Commercial media are thoroughly tested by the manufacturer and require either less QC testing or are categorized as generally "exempt" from user quality control according to the US Clinical and Laboratory Standards Institute (CLSI).

Recommendations for New Shipments of Media

Upon receipt of each new media shipment, check the following:

1. Manufacturer's QC records are provided - these records must be retained with the lab's QC records.
2. Expiration dates - notify the vendor of recurring short expiry dates.
3. Tubes or plates are not damaged or cracked.

4. The medium is not contaminated or changed in its appearance; e.g., color change with LJ media, blood agar hemolyzed, etc.
5. Agar is not detached from the sides of the tube/plate, frozen or softened.
6. Tubes/plates are sufficiently and equally filled.
7. Media is not excessively moist or dehydrated.

If any problems are found, notify supervisor, contact vendor immediately, and withhold media from patient use until issues are resolved.

QC of Commercial Media Inventory

It is important to monitor the performance of commercial media closely during use, and if necessary, perform complete quality control to ensure the recovery of isolates is satisfactory and as expected.

QC of Commercial LJ Media

Commercial LJ media has been demonstrated to deteriorate over time, and its ability to support growth can be compromised. Therefore, commercially-sourced LJ media that has been stored in the lab ≥6 weeks should be tested according to the sterility/performance checks in **Section** 16.2.1.2.1 QC Protocol for LJ Medium.

QC of Commercial MGIT Medium

Becton Dickinson (BD), the manufacturer of MGIT reagents, recommends QC testing of BACTEC MGIT medium and MGIT 960 Growth Supplement upon receipt (before putting into routine use), to ensure that the performance characteristics of the medium, once supplemented with the OADC/PANTA mixture, are acceptable. At a minimum, the Sponsor requires the QC procedure outlined in **Section** 16.2.1.3.1 below for new lots/shipments of MGIT culture medium using an *M. tuberculosis* strain. Refer to the MGIT culture media package insert for further instructions on QC procedures with non tuberculous mycobacterial strains.

QC of Commercial Blood Agar Plates

Contamination can sometimes occur during transit of commercial BAP media. Monitor each lot closely, and if contamination issues are suspected, follow the procedure as recommended in **Section** 16.2.1.2.3 above.

16.2.1.3.1 QC Protocol for BACTEC MGIT 960 Culture Medium and MGIT 960 Growth Supplement Kit

1. *Frequency:* Each new lot or new shipment of 960 media or growth supplement.
2. *Controls:* Dilutions of *M. tuberculosis* (H37Rv or H37Ra) in 7H9 broth.
3. *Acceptable results:* MGIT tube will fluoresce positive (TTD) in 6 to 10 days
4. *Corrective actions:*
   a. If the TTD is not within the specified range, repeat the test.
   b. If test still does not give satisfactory results upon repeat, notify supervisor immediately, and check the viability of the inoculum, age of the culture, if stored frozen, and other procedures.
      i. If all procedures are within established specifications, contact Technical Services at BD Diagnostic Systems for assistance and withhold use of lot.
5. *Documentation:* Record results on the Reagent/Media QC Form – Appendix E. If QC results are not acceptable, prepare an Appendix K form to document the corrective action.

Preparation of Culture Suspension

1. Subculture the MTB strain onto several LJ slants.
2. Incubate the tubes at 37ºC (±1°C), and observe growth visually.
3. Use colonies showing good, confluent, and pure growth within 10-15 days of first appearance. Younger or older cultures may not give reliable results.
4. Remove growth from the slant by carefully scraping the colonies off the slant with a sterile loop or sterile wooden applicator stick.
   → Take extreme precaution not to scrape off any culture medium (which gives false turbidity measurement).
5. Transfer growth into a screw cap tube containing 4 ml of sterile 7H9 broth and glass beads (6-10 beads, 2 mm diameter), which help to break up clumps (**Tube A**).
6. Vortex **Tube A** for at least 1-2 minutes, making sure the suspension is well dispensed and very turbid.
   → Turbidity should be greater than McFarland No. 1 standard.
7. Let the suspension stand undisturbed for 20 minutes.
8. Using a transfer pipette, carefully transfer the supernatant from **Tube A** to another sterile screw cap glass tube (**Tube B**).
   → Avoid pipetting any sediment.
9. Let **Tube B** stand undisturbed for 15 minutes.
10. Carefully transfer the supernatant from **Tube B** into another screw cap glass tube (**Tube C**) without taking any sediment.
11. Adjust the turbidity of the suspension in **Tube C** to a McFarland No. 0.5 standard by adding more 7H9 broth. Mix well.
    → If the suspension is too turbid, transfer some of the suspension to another sterile tube and adjust the turbidity to a McFarland No. 0.5 standard.
12. **Tube C** is the stock suspension for QC testing.
    → This suspension may be frozen in small aliquots (~1.5 ml) in cryotubes at -70 to -80ºC (±10ºC).
    → The frozen suspensions may be used for up to six months.
    → Once thawed, do not refreeze.
13. Ideally, ensure that new frozen stock passes testing indicated below before using in routine QC procedures.

Preparation of Dilutions

1. Remove one aliquot of the stock suspension from the freezer and allow to thaw.
2. Alternatively, prepare a fresh 0.5 McFarland suspension, using colonies within 10-15 days of first appearance from LJ media. Carefully adjust the inoculum with a spectrophotometer or visually with a Wickerham card.
3. Dilute the stock suspension (freshly prepared or frozen) 1:5 by transferring 1.0 ml of suspension to 4.0 ml of sterile water or saline. Mix well (**Tube 1**).
4. Dilute 1:10 by adding 0.5 ml of suspension from Tube 1 into 4.5 ml of sterile water or saline (**Tube 2**).
5. Mix well and then dilute 1:10 again by adding 0.5 ml from Tube 2 to 4.5 ml of sterile water or saline (**Tube 3**). Mix well.
6. The final dilution of **Tube 3** is 1:500.

Inoculation and Incubation

1. Supplement MGIT medium with Growth Supplement and PANTA as specified in **Section** 10: Liquid Culture – Mycobacteria Growth Indicator Tube (MGIT).
2. Inoculate 0.5 ml from **Tube 3** to a MGIT tube. Mix well.
3. Enter the inoculated tube in the MGIT 960 instrument.
4. Remove the tube when indicated positive by the instrument.
5. Retrieve data for time to detection from the MGIT printout.

16.3 Reagent Quality Control

Periodic quality control of all reagents is critical for ensuring confidence in laboratory results. For the clinical trial, the Sponsor requires the following reagent quality control procedures for staining reagents, sputum digestion/decontamination reagents, immunochromatographic identification tests and extraction buffer, and drug susceptibility reagents. In addition, record reagent name, batch number, date prepared, and expiry date on all reagent containers.

16.3.1 Acid-fast Stains: Fluorescent and Ziehl-Neelsen Methods

1. *Frequency:* Each batch of patient tests and each new batch of in-house prepared reagents, or each new lot/new shipment of commercial reagents.
2. *Controls:* Smears with known positive *M. tuberculosis* (H37Rv or H37Ra) and negative (non-acid-fast bacteria) control organisms.
3. *Acceptable results:* Correct results as expected for positive and negative controls.
   a. Positive controls must demonstrate the presence of acid-fast bacilli.
   b. Negative controls must be clearly negative with no acid-fast bacilli present.
4. *Corrective Actions:* If either control result is unacceptable, do not report patient results.
   a. Repeat controls; if acceptable, repeat patient tests.
   b. If results still unacceptable, notify supervisor immediately and prepare new reagents and/or new controls, as applicable, to resolve issue.
   c. When QC results are acceptable, repeat patient tests and report results.
5. *Documentation:* Record results for new staining reagents on the Reagent/Media QC Form – Appendix E. For each batch of smears, record results on the appropriate Daily AFB Staining QC Form – Appendix C. If QC results are not acceptable, prepare an Appendix K form to document the corrective action.

Preparation of Smears

Prepare a batch of positive and negative control smears in advance, heat-fix, and store unstained in a closed container in a dry area until used. It is preferable to use a seeded sputum sediment to prepare these controls, but if not available, a suspension of *M. tuberculosis* (H37Rv or H37Ra) for the positive control and *Escherichia coli* (or another non-acid-fast bacillus) for the negative control should be used. The *E. coli* suspension may be prepared from the stock culture used in Section 16.2.1.2.3: QC Protocol for Blood Agar Medium.

16.3.2 Sputum Digestion/Decontamination Reagents

For each new batch of the NALC, NaOH, and Na citrate reagents, record preparation details. Quality control for these reagents is performed as part of Quality Monitoring of Sputum Processing, below. Additional QC for the phosphate buffer is performed as follows:

1. *Frequency:* Each new batch of phosphate buffer.
2. *Controls:* pH paper or pH meter.
3. *Acceptable results:* pH for phosphate buffer is 6.8.
4. *Corrective Actions:* If pH for buffer cannot be adjusted to 6.8, discard batch, notify supervisor immediately, and prepare new batch using new reagent powder, if necessary.
5. *Documentation:* Record reagent details and pH on Reagent/Media QC Form – Appendix E. If QC results are not acceptable, prepare an Appendix K form to document the corrective action.

16.3.3 Immunochromatographic Identification Tests

1. *Frequency:*
   a. Each new lot or shipment of kits and each new prepared lot of extraction buffer.
   b. Weekly, or with each batch of patient tests, if testing is performed less frequently.
2. *Controls:*
   a. Internal reagent control in device.
   b. Positive control: Culture of *M. tuberculosis* reference strain (H37Rv or H37Ra) in MGIT broth.
   c. Negative control: Culture of a MOTT strain (e.g., a well characterized strain of *M. avium* complex) in MGIT broth or broth from an uninoculated MGIT tube.
3. *Acceptable results:* Correct results as expected for all controls.
   a. Internal control line is visible.
   b. *M. tuberculosis* must result in a positive test.
   c. MOTT strain or uninoculated broth must result in a negative test.
4. *Corrective actions:* If any control result is unacceptable, do not report patient tests.
   a. Repeat test with new controls; if acceptable, repeat patient tests.
   b. If repeat results still unacceptable, notify supervisor immediately and investigate potential causes for failure.
   c. After investigation is complete and QC is acceptable, repeat patient tests and report results.
5. *Documentation:* Record results for new extraction buffer on Appendix E; Record results for new kits on MTB Identification QC Form – Appendix G. If QC results are not acceptable, prepare an Appendix K form to document the corrective action.

Procedure

1. Follow procedures outlined in **Section** 13 to test controls.
2. Perform QC tests immediately before testing the patient batch and resolve issues, if applicable.

16.3.4 Drug Susceptibility Testing Quality Control

1. *Frequency:*
   a. Upon receipt of a new lot or new shipment of MGIT 960 SIRE kit, PZA kit, or PZA tubes.
   b. Upon receipt of a new lot, new shipment, or newly prepared batch of stock solutions of antibiotic powders for second-line drugs.
   c. Weekly or with each batch of patient testing.
2. *Controls:* A pan-susceptible *M. tuberculosis* H37Rv or H37Ra strain
3. *Acceptable results:* Susceptible results for all drugs within the defined time protocol; e.g., SIRE and second-line drugs within 4-13 days; PZA within 4-21 days.
4. *Corrective Actions:* If the control results show any resistance, do not report patient results for that particular drug(s).
   a. Thoroughly review cause for unacceptable results and repeat QC test; if acceptable, repeat patient tests.
   b. If repeat results still unacceptable, notify supervisor immediately; prepare new drugs and/or a new control, as applicable, to resolve issue.
   c. If unacceptable results persist with SIRE or PZA kit, consult BD Technical Services for assistance.
   d. When QC results are acceptable, repeat patient tests and report results.
5. *Documentation:* Record results for new antibiotics and weekly testing on the DST QC Form – Appendix F. If QC results are not acceptable, prepare an Appendix K form to document the corrective action.

Inoculum Preparation

The inoculum must consist of a pure culture of *M. tuberculosis* (H37Rv or H37Ra) and be tested following the same procedures as for patient isolates. The inoculum can be prepared from one of three options:

1. A freshly grown culture of MTB in MGIT medium following the guidelines in **Section** 12.4: Inoculum for MGIT DST.
2. A fresh, pure subculture of MTB growing on LJ medium following the guidelines in **Section** 12.4.2: Using an Inoculum from Positive LJ Culture.
3. A suspension of MTB prepared according to steps 1-12 in **Section**16.2.1.3.1: QC Protocol for BACTEC MGIT 960 Culture Medium and MGIT 960 Growth Supplement above.
   a. Remove one aliquot of the organism suspension from the freezer and allow to thaw.
   b. Dilute 1 ml of the suspension in sterile saline or 7H9 broth (1:5 dilution).
   c. Use this adjusted suspension as the inoculum for drug tests and growth control.

Set-up of DST Quality Control

Follow instructions in **Section** 12.5: Growth Control Tube Preparation and Inoculation for the drugs being tested. Important considerations when preparing the DST QC are:

→ Proper reconstitution of lyophilized drugs.
→ Proper preparation of drug powder suspensions.
→ Proper dilution of the QC organism for the Growth Control and drug tubes.

Monitor QC drugs until signaled complete by the instrument and interpret the same as for patient isolates.

16.4 Quality Monitoring Activities

Monitoring of critical practices that may affect the outcome of clinical trial results is **required** for the duration of the study. These practices include:

1. Temperature monitoring
2. Equipment maintenance
3. Regular monitoring of specimen processing
4. MGIT time to detection (TTD) monthly exercise
5. Contamination rate assessment
6. Supervisor review of QC activities on a monthly basis

In addition, though not required, the following quality monitoring activities are **strongly recommended** and will serve to strengthen laboratory results:

1. Review of AFB examination competency
2. Analysis of laboratory data

16.4.1 Temperature Monitoring (Required)

16.4.1.1 Thermometers

Internal thermometers must be used for all equipment monitoring. All thermometers used in assessing equipment temperatures must be calibrated against a standardized/certified thermometer (US Bureau of Standards or equivalent) before putting into use and annually thereafter, either in-house or through a commercially available service. Identify thermometers by a numbering system, and maintain documentation of their calibration in a lab worksheet or logbook.

Table 16.3 Equipment Temperature Ranges

Equipment	Temperature Ranges (°C)
Refrigerators[a]	2-8
Freezer (Ultralow)	–70 to -80 ±10
Incubators	37±1
Refrigerated centrifuge	4-12
MGIT 960	37±1[b]
Room Temperature[c]	varies

a Temperature of the refrigerator where sputum specimens are stored until they are sent to the laboratory must be monitored daily.
b Internal instrument temperature reading should be ± 1.5°C of manual reading.
c Range determined by requirements of reagents or instrument housed in the particular room; e.g., MGIT room: 19-30°C; room containing unrefrigerated centrifuge: ≤ 20°C.

16.4.1.2 Temperature Reading/Recording

1. *Frequency:*
   a. Daily reading/recording of temperatures is required, preferably in the early morning before work commences. Twice daily readings are *recommended* as equipment may malfunction during working hours, which may not be detected until the following day.
   b. Weekends/holidays may be excluded if no staff are available to monitor the equipment during those times; check temperatures immediately the beginning of the next working day.

2. *Acceptable results:* Temperatures are within the defined range for the specific piece of equipment/environment.
3. *Corrective actions:* If the temperature reading is not acceptable:
   a. Adjust temperature control and monitor until correct range is achieved; if temperature has not returned to normal within the next working day, notify supervisor immediately to decide on further corrective action.
   b. If equipment is not functioning, notify supervisor immediately so service can be instituted.
      i. Relocate specimens, reagents, etc., as applicable, to a functioning piece of equipment/environment with proper temperature range.
4. *Documentation:*
   a. Record temperatures on Appendix D: Equipment Temperature Record Form.
   b. Document minor adjustments and corrective actions on Appendix D.
   c. Document all major unacceptable results, e.g., equipment failure, corrective actions and resolutions on an Appendix K form.

16.4.2 Equipment Cleaning/Maintenance (Required)

16.4.2.1 Equipment cleaning

Keeping laboratory equipment clean and performing recommended routine maintenance are essential for accurate performance of laboratory tests and maintaining the longevity of equipment. Suggested equipment cleaning activities are given in the table below, however the manufacturers' specific recommendations and procedures should be followed. Maintain documentation for the performance of routine cleaning in a lab worksheet or logbook.

Table 16.4 Equipment Cleaning Schedule

Equipment	Cleaning	Daily	Weekly	Monthly	Other
Biosafety cabinet	Spray work surfaces thoroughly with tuberculocidal disinfectant; let stand 3 minutes. Wipe dry with absorbent towel. If indicated, follow with 70% alcohol to remove disinfectant residue that may harm BSC surface. Wipe equipment stored in the BSC with disinfectant soaked absorbent towel.	AM & PM and following each task			
	Thorough interior and exterior cleaning with appropriate disinfectant(s)				At least once/year or as needed for any spill of infectious material
	Clean UV lights with 70% alcohol				Every 1-2 weeks
Centrifuge	Spray interior walls of centrifuge with disinfectant (70% alcohol) and let stand 3 minutes. Wipe dry with absorbent towel. Clean exterior surfaces with mild detergent, rinse and dry.	X			
	Remove carriers and soak in warm, soapy water. Rinse thoroughly and place upside down to drain/dry.		X		
Counters	Spray thoroughly with appropriate tuberculocidal disinfectant; let stand 3 minutes. Wipe dry with absorbent towel. If indicated, follow with 70% alcohol to remove disinfectant residue.	AM & PM			
–70 to –80°C Freezer	Clean fan cover and remove ice buildup			X	
	Defrost and clean interior				When needed
Micropipettors/ Pipette-Aid	Wipe exterior with 70% alcohol	Before/after each procedure use			
pH Meter	Replace electrode immersion fluid (pH 7.0 standard). Clean electrode tip				As needed
Refrigerator	Wipe interior surfaces and shelves with damp towel soaked in mild detergent; rinse and dry.			X	
Incubator	Wipe interior surfaces and shelves with damp towel soaked in mild detergent; rinse and dry. Follow with 70% alcohol spray. Autoclave shelves if applicable.			X	
Slide warmer	Wipe surface with a damp towel soaked in mild detergent; rinse and dry.		X		
Vortex mixer	Wipe surface with a damp towel soaked in mild detergent; rinse and dry.		X		
Analytical balance	Clean pan surface with brush.	X			

16.4.2.2 Equipment Maintenance

Routine, in-house maintenance must be carried out at the time intervals recommended by the manufacturer of the equipment, and a schedule developed for performing the applicable maintenance (Table 16.5). Maintenance may be performed by in-house technical personnel and/or a vendor service contract Maintain documentation of all routine maintenance in a lab worksheet or logbook.

A suggested maintenance schedule performed in-house or by outside technical personnel, as applicable, is shown below. Semi-annual or annual maintenance is required for all equipment in this list. In addition, if large equipment is moved/ relocated, it must be re-certified or serviced prior to using.

Table 16.5 Minimum Equipment Maintenance Schedule[1]

Equipment	Maintenance	Semi-annually	Annually
Autoclave	Maintenance by autoclave technician	recommended	required
Micropipettors/ Pipette-Aid	Calibration	recommended	required
Analytical balance	Calibration	recommended	required
Biosafety cabinet	Certification	–	required
Centrifuge	Calibration of speed, timer, temperature	recommended	required
Microscopes	Inspection, cleaning, and lubrication by service provider	recommended	required
Air conditioners	Clean condensers, fans and blower motor; verify mechanics and check filter	recommended	required
Freezer -70 to -80°C	Clean condensers; verify door gaskets	recommended	required
	Clean fan and blower motor; routine maintenance	recommended	required
Refrigerators	Clean condensers and fans; routine maintenance	recommended	required
Incubator	Check door gasket seal, heating and cooling elements, electronic components; routine maintenance	recommended	required
MGIT	Calibration tube check, thorough cleaning of relevant components	required	–

[1] For further details on equipment maintenance and cleaning, refer to: Maintenance Manual for Laboratory Equipment, 2nd Edition. World Health Organization. Geneva, Switzerland. 2008.

16.4.3 Monitoring of Sputum Processing (Required)

Careful attention to technique when processing specimens is essential to preventing cross-contamination from a heavily acid-fast positive specimen to other, possibly negative, samples. Running positive and negative controls on a regular basis assesses techniques and assures that all aspects of the culture process (manual and instrumented), from sputum processing to isolation, are performing properly.

16.4.3.1 Quality Monitoring of Sputum Processing

1. *Frequency:* Once per week, or with each patient batch.
2. *Controls:* Place controls at the end of the batch of patient tests, with the positive control before the negative control. Process both control specimens in the same manner as patient specimens and perform routine microscopy, MGIT, and LJ culture.
   a. Positive control – 4 ml 7H9 Broth, or a known negative sputum specimen, inoculated with 700 µl of a 10^{-2} dilution of a McFarland No. 0.5 suspension of *M. tuberculosis* (H37Rv or H37Ra). Suspension should be prepared using 10–15 day old growth on LJ. Refer to **section** 16.2.1.3.1 for details.
   b. Negative control: 4 ml 7H9 broth.
3. *Acceptable results:*
   a. Positive control
      i. Positive fluorescent smear.
      ii. Growth in MGIT; TTD should be comparable with similar tests.
      iii. Growth on LJ culture; colony counts should be comparable with similar tests.
   b. Negative control
      i. Negative fluorescent smear.
      ii. No growth in MGIT or on LJ culture.
4. *Corrective actions:* If QC results are unexpected, notify supervisor immediately and have technician repeat exercise with next processing batch.
   a. If growth is detected on either media from the negative control, either cross-contamination has occurred or there is a problem with sterility of the processing reagents. **Notify the Sponsor immediately if cross-contamination of MTB is suspected.**
      i. Observe the technician who processed these specimens for techniques known to contribute to contamination; e.g., poor organization of tubes, splashing when adding reagents, opening caps too soon after vortexing, etc.
      ii. Visually check all reagents used; subculture to BAP if contamination is suspected.
      iii. Record patient samples processed at this time and monitor closely for expected results.
   b. If no growth occurs on either media from the positive control, check age of isolate used and diluting technique.
   c. If TTDs and/or colony counts on LJ vary widely, investigate possible cause for deviations.
5. *Documentation:* Record the microscopy, MGIT and LJ culture results on the Weekly MGIT/LJ Culture QC Form – Appendix H. If QC results are not acceptable, prepare an Appendix K form to document the corrective action.

16.4.4 MGIT 960 Time To Detection Monthly Exercise (Required)

An important control measure of the MGIT culture system is to evaluate the consistency (reproducibility) of the TTD for known quantities of *M. tuberculosis* organisms. This exercise assesses consistency of technical performance in preparing MTB suspensions according to a turbidity standard, diluting MTB suspensions, and pipetting skills.

1. *Frequency:* Once per month, and performed by alternating technicians, if appropriate.
2. *Controls:* One dilution of *M. tuberculosis* (H37Rv or H37Ra) in 7H9 broth. It is highly recommended that a frozen stock, no older than six months, be used to prepare the dilution.

3. *Acceptable results:*
   a. The TTD must fall within the 6-10 day range as referenced in the FIND MGIT Procedure Manual.
   b. Month to month consistency in the lab's established TTD range for the 1:500 dilution.
4. *Corrective actions:* TTD results will be closely monitored by comparing each monthly TTD result to the laboratory's own ongoing range of results and to a global range established by the Sponsor laboratory network. If expected results vary by more than ± 2 standard deviations within the laboratory's own established range:
   a. Review data and all procedures with technician performing the exercise and repeat test under observation if necessary.
   b. If TTD is significantly decreased, check culture for contamination and, if contaminated, repeat exercise with fresh culture.
   c. If TTD is significantly increased, check viability of culture and age of culture (if using frozen stocks).
5. *Documentation:*
   a. Record results on the MGIT TTD Worksheet – Appendix L
   b. If QC results are not acceptable, prepare an Appendix K form to document the corrective actions.
   c. Each month upon test completion, provide completed Appendix L form, along with applicable MGIT printouts and any Appendix K forms, to the Sponsor. File copy of documents in laboratory QC binder.

Preparation of Culture Suspension

Follow instructions in **Section** 16.2.1.3.1: QC Protocol for BACTEC MGIT 960 Culture Medium and MGIT 960 Growth Supplement Kit to prepare the culture suspension.

Preparation of 1:500 Dilution

NOTE: It is highly recommended to prepare the dilution from a frozen stock suspension.

1. Remove one aliquot of the stock suspension from the freezer and thaw. Alternatively, prepare a fresh McFarland No. 0.5 suspension using colonies within 10-15 days of first appearance on LJ media. Carefully adjust the inoculum with a spectrophotometer or visually with a Wickerham card.

2. Dilute the stock suspension (freshly prepared or frozen) 1:5 by transferring 1.0 ml of suspension to 4.0 ml of sterile water or saline. Mix well (**Tube 1**).

3. Dilute 1:10 two more times by adding 0.5 ml of suspension from **Tube 1** into 4.5 ml of sterile water or saline (**Tube 2**). Mix well, and then again add 0.5 ml from **Tube 2** to 4.5 ml of sterile water or saline (**Tube 3**).

Tube 3 will be used for the inoculation and incubation of the MGIT tube. This tube represents a 1:500 dilution, representing about $\log 10^5$ organisms.

Inoculation and Incubation

1. Supplement MGIT medium with Growth Supplement and PANTA as specified in **Section** 10: Liquid Culture – Mycobacteria Growth Indicator Tube (MGIT).

2. Inoculate one MGIT tube with 0.5 ml of the 1:500 dilution (**Tube 3**).

3. Enter the inoculated tube in the MGIT 960 instrument. Take the tube out when indicated positive by the instrument. Retrieve data for time to detection.

16.4.5 Contamination Rate Assessment (Required)

A monthly assessment of the rates of contamination in MGIT and LJ cultures demonstrates trends of increasing or decreasing contamination and should be monitored.

1. *Frequency:* Once a month
2. *Results:* Contamination of liquid and solid cultures occurs:
   a. When specimens are inadequately decontaminated because specimens are heavily mucoid or have been improperly stored during transport encouraging bacterial overgrowth.
   b. Some highly resistant bacterial species are unaffected by decontamination.
3. *Corrective Actions:* Reasonable rates of contamination are unavoidable.
   a. If MGIT contamination rate is >10%, notify the Sponsor immediately for guidance and troubleshooting instructions.
   b. If LJ contamination rate is >6%, notify the Sponsor immediately for guidance and troubleshooting instructions.
4. *Documentation:* Report findings on Monthly Data Monitors Form – Appendix I.

Procedure

1. Count the total number of MGIT cultures reported in the month and the number of contaminated cultures reported in that month. Calculate the percentage (%) contaminated (number contaminated ÷ number reported × 100 = % contamination).
   NOTE: Using this method, the cultures processed in any given month may not correlate exactly with the cultures reported in that same month, but statistics over time will provide consistent indicators for the overall performance of lab procedures.
2. Repeat Step 1 for LJ cultures.
3. Repeat steps 1 and 2, separating patient-collected specimens and hospital/clinic-collected specimens for both MGIT and LJ cultures.
4. Record the % contaminated for each medium and compare results with data from the previous month and the lab's average.
5. Calculate the on-going cumulative mean rate for each medium.

16.4.6 AFB Smear Review for Technical Competency (Strongly recommended)

Periodic smear review is recommended to assess and improve technician proficiency and assure consistency among examiners.

1. *Frequency:* Once a month
2. *Acceptable results:*
   a. Negative smears should be resulted as negative by all technicians.
   b. Positive smears should not vary by more than one quantification level.
3. *Corrective actions:*
   a. Review discrepant results with technician(s) and re-examine smears that are not in agreement.
   b. Provide additional training as indicated to improve performance.
4. *Documentation:* Save results in QC binder and technicians' personnel files, if appropriate.

Procedure

1. Select 10 slides from those read in the previous month and that represent a range from negative to scanty, 1+, 2+, and 3+.
2. Ask technicians to read and record results in a blinded manner.
3. Review results for consistency and any discrepancies among technicians.

16.4.7 Analysis of Laboratory Data (Strongly recommended)

It is strongly recommended that laboratory data be recorded and analyzed to help assure that specimen processing procedures, as well as mycobacterial isolation rates, are performing properly. After initially analyzing the records for three to six months, an overall average or normal trend for an individual laboratory workload can be determined. Monthly Data Monitors Forms – Appendix I is provided for recording these data.

If there is significant change or deviation from normal results in any of the parameters, all procedures must be reviewed and corrective measures instituted.

Frequency: Each parameter should be calculated monthly.

Culture Positivity Rate

1. *Procedure:*
   a. Count the number of positive MGIT cultures that are smear positive. Count the total number of positive MGIT cultures. Use these values to calculate the MGIT isolation rate from smear-positive specimens (smear positive ÷ total culture positive *reported* in the month x 100).
   b. Count the number of positive MGIT cultures that are smear negative. Count the total number of positive MGIT cultures. Use these values to calculate the MGIT culture positivity from smear negative specimens (smear negative ÷ total culture positive *reported* in the month x 100).
   c. Repeat Steps a. and b. for LJ cultures.
   d. Record isolation rates and compare results with data from the previous month and the lab's average.
   e. Calculate the cumulative mean positivity rate for each medium.
2. *Results:*
   a. > 90% of smear positive specimens should be culture positive.
   b. 50% of culture positive specimens may be from smear-negative specimens.
   c. Rates should be relatively consistent for each medium. Due to the higher sensitivity of liquid medium, the isolation rate on LJ may be slightly lower, especially if smears show very few AFB.
3. *Corrective Actions:*
   a. If there is a significant decrease in overall culture positivity (isolation) rate for both media types, review decontamination procedures.
   b. If there is a significant decrease in only one media type, check the growth performance, following media QC guidelines above.
   c. If the LJ isolation rate differs by >20% of the MGIT isolation rate for the same set of specimens, perform QC procedures for the applicable lot of LJ (commercial or homemade), following the guidelines in **Section** 16.2.1.2.1: QC Protocol for LJ Medium.
   d. If there is a significant change in smear positivity/negativity, unrelated to the visit intervals examined, review all microscopy procedures and discuss with personnel.

4. *Documentation:*
   a. Record data on the Monthly Data Monitors Forms – Appendix I – or other comparable lab-specific document.
   b. If any corrective measures are necessary, prepare an Appendix K form to document the activities.

Isolation Rate of MTB and MOTT

1. *Procedure:*
   a. Count the number of MTB isolates and calculate the percentage using the total MGIT cultures *reported* in a given month (MTB culture ÷ total culture positive x 100).
   b. Count the number of MOTT isolates and calculate the percentage using the total MGIT cultures *reported* in a given month (MOTT culture ÷ total culture positive x 100).
   c. Repeat steps a. and b. for LJ cultures.
   d. Record isolation rates for both species and compare results with data from the previous month and lab's average.
   e. Calculate the cumulative mean positivity rate for each mycobacterial species.
2. *Results:* Isolation of MTB complex organisms in relation to other mycobacterial species should remain fairly constant over time. If there is a sudden increase in isolation of MOTT, it may be due to increase in numbers of follow-up specimens from subjects colonized with MOTT (a common observation). However, this may also indicate the presence of an environmental contaminant or cross-contamination event.
3. *Corrective Actions:*
   If there is a significant increase in MOTT isolation, seemingly unrelated to individual patient colonization, thoroughly review all procedures (e.g., safety, cleaning, specimen processing, culture media, etc.), using supervisor observation whenever necessary.
4. *Documentation:*
   a. Record data on the Monthly Data Monitors Forms – Appendix I – or other lab-specific comparable document.
   b. If any corrective measures are necessary, prepare an Appendix K form to document the activities.

Time to Detection

1. *Procedure:*
   a. Calculate the time to detection for MGIT cultures from smear positive and smear negative specimens that are determined to be **MTB**.
   b. Repeat step a. for LJ cultures.
   c. Record results for both media and compare results with data from the previous month and lab's average.
   d. Calculate the cumulative average TTDs for each medium type.
2. *Results:* If the monthly average TTD is <20% of the lab's established average (without an increase in contamination rate or isolation of rapidly growing mycobacteria), this likely indicates that processing has improved. If the monthly average TTD >20% of the lab's established range, processing of specimens may be too harsh or centrifuge speed/time may be less than optimal.
3. *Corrective Actions:*
   a. If there is a significant increase in TTD (unrelated to visit interval), review processing procedures and discuss with personnel, using supervisor observation if necessary.
   b. Although an improvement in processing is generally considered beneficial, significant decreases in TTD may also prompt review of applicable procedures.
   c. Review settings and maintenance records for centrifuge.

4. *Documentation:*
   a. Record data on the Monthly Data Monitors Forms – Appendix I – or other lab-specific comparable document.
   b. If any corrective measures are necessary, prepare an Appendix K form to document the activities.

Technologist Assessment

All parameters described above should remain similar among different technologists responsible for processing specimens. Tracking lab personnel along with their specimen responsibilities is helpful in the event that an unusual episode of contamination or cross-contamination occurs, or when overall positivity rates and other parameters deviate from the normal range.

1. *Procedure:*
   a. Record the names of technologists performing procedures on the laboratory's processing worksheet.
   b. Record the lab accession numbers of specimens processed in each batch.
2. *Results:* When conducting reviews on identified deviations in the parameters above, check for any correlation with a specific technologist(s), which could indicate possible deviations from standard processing protocols.
3. *Corrective Actions:*
   a. If reviews implicate a specific staff member, re-train the technologist as appropriate. Ideally, review each step of the specimen processing SOP with the technologist(s) on a frequent (quarterly) basis, and especially before each change in rotation.
   b. Perform annual competency assessments of all technologists to control for technician-related deviations.
4. *Documentation:*
   a. Document all lab trainings, re-trainings and competency assessments on the laboratory-specific form.
   b. If any corrective measures are necessary, prepare an Appendix K form to document the activities.

16.5 Quality Improvement

Continuous efforts to improve the overall quality of the laboratory, by improving service, function, workflow, personnel, and customer satisfaction are necessary and require the input and support of all personnel. Regular staff meetings should be held to share and discuss quality issues found with QC and QM, to elicit suggestions for improvement, and encourage input from staff on any issues of concern.

The Sponsor requires the documentation of efforts to improve the quality of the lab. When Quality Control tests fail to give the proper results and/or when deviations from baseline data are observed, the use of a standardized form, such as Appendix K, must document the action taken to correct the problem. The form includes the following components:

- Form Number - number assigned to each form to allow easy filing and retrieval. Often the date is incorporated into this number.
  Ex: Form Number: 01JAN11-a

- Description of the problem/improvement - The date and a short description are written here.
  Ex: Monthly TTD QC exercise not performed in January 2011. Problem discovered 30JAN11.

- Investigation into the root cause of the problem - Oftentimes, there is a bigger underlying problem that causes a QC failure or deviation to occur. The issue should be analyzed, and the root cause written in this section.
  Ex: TTD QC exercise was not assigned to a particular laboratorian.30JAN11.

- Corrective action to fix the current problem - The first action taken to resolve the immediate issue is written here, along with the expected date of resolution.
  Ex: TTD QC exercise performed immediately on 30JAN11.

- Preventative action required to eliminate root cause of problem - This action often differs from the action taken to fix the immediate problem (mentioned above), and should address the root cause of the problem.
  Ex: A schedule was prepared to ensure each MGIT user performs the TTD exercise. 01FEB11.

- Person(s) responsible for monitoring the effectiveness of action - This section should explain how the effectiveness of the previously mentioned action is evaluated.
  Ex: Ms. Pim was assigned to check the schedule in March, and verify the appropriate laboratorian signed by their name after performing the TTD QC exercise for both February and March. 21MAR11.

- All persons involved in the incident, e.g., the person identifying the issue, the QA delegate and the lab supervisor, should review the form and sign the document, attesting that s/he has been notified of the issue and agree to the actions taken.

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17. World Health Organization. Maintenance Manual for Laboratory Equipment, Second Edition. Geneva, Switzerland. 2008. www.who.int/diagnostics_laboratory

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APPENDIX A: SPECIMEN TRANSFER FORM

Attach a study label to this page. This form should accompany any specimen generated by a patient enrolled in the Sponsor study to the laboratory. Use a separate Appendix A form for each sample submitted. Once the form is completed, it should remain in the lab files.

Dispatch Section

This section should be completed by Principal Investigator or delegate.

Site name					Site #									
Screening ID #			Subject ID #				PT initials (3 characters)			....				
Visit # (circle one)	2	3	4	5	6	7	8	9	10	11	12	13	14	
	15	16	17	18	19	20	21	22	23	24	25	26		
	27	28	29	30	31a	31b	32a	32b	33	34	ET	UNS		
Date*/time collected (or received, if patient-collected)	..... / ..... / .... : ..... (24h clock)													
Specimen Number (circle one - only circle N/A if Visit 2 or 3)	☐ 1 ☐ 2 ☐ N/A				Sputum induced?			☐ Yes ☐ No						
Specimen collected by: (circle one)	☐ patient ☐ site staff				Specimen volume			..... ml						
Time at room temp[1]	..... hr ..... min ([1]Record 0 hr 0 min if immediately refrigerated or shipped)				Attach study label here:									
Date*/Time of dispatch to laboratory	..... / ..... / .... : ..... (24h clock)													
Mode of transport (circle one)	☐ in-person		☐ courier		☐ lab messenger		☐ other:							
Name of person collecting (or receiving, if patient-collected) specimen														
Name of person completing this form					Signature									

Transport Section

This section should be completed by the driver/courier.

Specimens refrigerated or kept on ice? (tick one)	☐ Yes ☐ No ☐ Unknown			
Driver name or name of courier		Signature		

Laboratory Section

This section should be completed by the laboratory technician receiving the specimen.

Date*/time specimen received	..... / ..... / .... : ..... (24h clock)		
Specimens refrigerated or kept on ice upon receipt? (tick one)	☐ Yes ☐ No	Lab accession number	
Sample in good condition, i.e. container is intact and properly labeled, no leakage, specimen at proper temperature? (tick one)	☐ Yes ☐ No		
If no, give details		Is study label missing?	☐ Yes ☐ No
Name of technician		Signature	
*Date format dd/mmm/yyyy Comments:			

APPENDIX B: STUDY SOURCE DOCUMENT WORKSHEET

Lab Accession #		Site #		Patient Initials	…… …… ……
Screening ID #		Subject ID #		Visit #	
Sputum Specimen	1 ☐ 2 ☐ N/A ☐ (Only tick N/A for V2 or V3)		Affix a completed study specimen label here:		

Sputum specimen

Received		Processed		Volume (ml)	Character (tick all boxes that apply)
Date (dd/mmm/yyyy)	Time (24hr clock)	Date (dd/mmm/yyyy)	Time (24hr clock)		
					Liquid / Saliva ☐ Blood ☐ Purulent ☐ Viscou ☐

Sputum induced?	Yes ☐ No ☐	Technician initials:	
Sputum collected by:	Patient ☐ Site ☐	Date:	
Comments:			

AFB microscopy from sputum specimen

Type of smear: (tick one box)	Auramine O ☐ Auramine/Rhodamine ☐ Acridine Orange ☐		Date smear read: dd/mmm/yyyy		Technician initials:	
Smear Result: (tick one box)	☐ Negative	☐ Rare	☐ Few	☐ Many	☐ TNTC	☐ Not Done (Comment required)
Comments:						

MGIT 960 system culture of sputum

Date of inoculation: (dd/mmm/yyyy)		**Original** TTD from MGIT printout:	Days	Hours	**Final** Time to Result (TTD) original or revised	Days	Hours
Date of MGIT result: (dd/mmm/yyyy)							
MGIT Culture Result: (tick one box)	☐ Negative for MTB complex ☐ Positive for MTB complex ☐ No TB growth, but positive for other mycobacteria		☐ Positive for MTB complex and contaminated ☐ Contaminated ☐ Unknown				
ZN Result: (tick one box)	☐ Negative for AFB ☐ Positive for AFB ☐ N/A	BAP Result: (tick one box)	☐ Contaminated and/or MOTT ☐ No growth ☐ N/A				
Identification of AFB	☐ Negative for MTB complex ☐ Positive for MTB complex ☐ N/A						
ID Test Method	☐ TBc ID ☐ Capilia ☐ Other: ……		Tech initials/date (dd/mmm/yyyy)				
Did specimen require re-decontamination?	☐ Yes ☐ No		Tech initials/date (dd/mmm/yyyy)				
Final date of MGIT culture completion (dd/mmm/yyyy)/Tech initials:							
Comments:							

AFB = Acid Fast Bacilli; BAP = Blood Agar Plate; ZN = Ziehl-Neelsen

Storage of Specimens

Was specimen banked for short-term storage on LJ medium?	☐ Yes	☐ No	☐ N/A	Was specimen banked for long-term storage at –70°C?	☐ Yes	☐ No	☐ N/A

Supervisor signature: …… Date: …… / …… / ……

My signature confirms that all data have been reviewed for accuracy and that all pre-filled information on this form is accurate for this specimen.

Screening ID #		Subject ID #		Visit #		Site #	
Sputum Specimen	1 ☐ 2 ☐ N/A ☐ (Only tick N/A for V2 or V3)						

LJ Culture of Sputum

Date of inoculation: (dd/mmm/yyyy)		Date of LJ result: (dd/(dd/mmm/yyyy)/yyyy)	
Result: (tick one box)			

☐ Negative for MTB complex	☐ TB growth (1-9 colonies)	☐ TB growth (10-100 colonies)	☐ TB growth (more than 100 colonies)	☐ TB growth (innumerable or confluent growth)	☐ No TB growth, but positive for other mycobacteria	☐ Contaminated	☐ Positive for MTB complex and contaminated	☐ Unknown

Confirmatory ZN Stain Results: (Tick one box)	☐ Negative for AFB	☐ Positive for AFB	☐ N/A
Identification of AFB	☐ Negative for MTB complex	☐ Positive for MTB complex	☐ N/A

ID Test Method:	☐ TBc ID	☐ Capilia	☐ Other:	Tech initials/date: (dd/mmm/yyyy)	
Final date of LJ culture completion: (dd/mmm/yyyy)				Tech Initials	
Comments:					

Drug susceptibility testing ☐ Not Applicable or Not Required per Protocol

(**S** = Sensitive; **R** = Resistant; **TF** = Test Failed; **ND** = Not Tested)

Drug	Testing Method (MGIT, other- specify)	Date of Result (dd/mmm/yyyy)	Conc. (ug/ml)	S	R	TF	ND
Isoniazid	MGIT		0.1	☐	☐	☐	☐
Rifampicin	MGIT		1.0	☐	☐	☐	☐
Pyrazinamide	MGIT		100	☐	☐	☐	☐
Ethambutol	MGIT		5.0	☐	☐	☐	☐
Streptomycin	MGIT		1.0	☐	☐	☐	☐
Kanamycin				☐	☐	☐	☐
Amikacin	MGIT		1.0	☐	☐	☐	☐
Capreomycin	MGIT		2.5	☐	☐	☐	☐
Ofloxacin	MGIT		2.0	☐	☐	☐	☐
Moxifloxacin	MGIT		0.25	☐	☐	☐	☐
Levofloxacin	MGIT		2.0	☐	☐	☐	☐
Gatifloxacin				☐	☐	☐	☐
Enviomycin Sulfate				☐	☐	☐	☐
1st line DST Tech Initials/Date: (dd/mmm/yyyy)		2nd line DST Tech Initials/Date: (dd/mmm/yyyy)					
Comments:							

Shipping

Was/were isolate(s) shipped?		Yes ☐	No ☐	N/A ☐
Isolate	1 ☐ 2 ☐ N/A ☐ (Only tick N/A for V2 or V3)	If Yes, date of shipment (dd/mmm/yyyy)		
For V2 and V3 shipping only:				
Aliquot #1 Date of shipment: (dd/mmm/yyyy)		Aliquot #2 Date of shipment: (dd/mmm/yyyy)		
Comments:				

Supervisor signature: ..... Date: ..... / ..... / .....

My signature confirms that all data have been reviewed for accuracy and that all pre-filled information on this form is accurate for this specimen.

APPENDIX C: DAILY QC STAINING FORMS

Appendix C: Acridine Orange Daily QC Staining Form							
Month:						Year:	
Note: If QC is out of range, inform the supervisor and record any action taken below.							
Acridine Orange Batch or Lot #/ Expiration Date**		2N H_2SO_4 Batch or Lot #/ Expiration Date**		Acid Alcohol Batch or Lot #/ Expiration Date**			
Positive Control Slides date of preparation		Positive control slide date of expiration		Negative control slide date of preparation		Negative control slide date of expiration	

Day	Results (Neg, Confirmation Required*, Scanty, 1+, 2+, 3+)		QC Pass (Yes, No)	Technician Initials
	Positive control[1]	Negative control[2]		
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
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30				
31				

[1]MTB strain tested/ positive control		[2]Strain tested/negative control	
Reviewed by:			
Date**			
Signature:			
Comments:			

*Confirmation required by another technician or prepare another smear, stain and read. **Date format: dd/mmm/yyyy

Appendix C: Auramine O Daily QC Staining Form

Month: ..... Year: .....

Note: If QC is out of range, inform the supervisor and record any action taken below.

Auramine O Batch or Lot #/ Expiration Date**		Acid Alcohol Decolorizer Batch or Lot #/ Expiration Date**		Potassium Permanganate Batch or Lot #/ Expiration Date**	
Positive Control Slides date of preparation		Positive control slide date of expiration		Negative control slide date of preparation		Negative control slide date of expiration	

Day	Results (Neg, Confirmation Required*, Scanty, 1+, 2+, 3+)		QC Pass (Yes, No)	Technician Initials
	Positive control[1]	Negative control[2]		
1				
2				
3				
4				
5				
6				
7				
8				
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29				
30				
31				

[1]MTB strain tested/ positive control		[2]Strain tested/negative control	

Reviewed by:

Date**

Signature:

Comments:

*Confirmation required by another technician or prepare another smear, stain and read. **Date format: dd/mmm/yyyy

Appendix C: Auramine/Rhodamine Daily QC Staining Form

Month: ..... Year: .....

Note: If QC is out of range, inform the supervisor and record any action taken below.

Auramine/ Rhodamine Batch or Lot #/ Expiration Date**		Acid Alcohol Decolorizer Batch or Lot #/ Expiration Date**		Potassium Permanganate Batch or Lot #/ Expiration Date**	
Positive Control Slides date of preparation		Positive control slide date of expiration		Negative control slide date of preparation		Negative control slide date of expiration	

Day	Results (Neg, Confirmation Required*, Scanty, 1+, 2+, 3+)		QC Pass (Yes, No)	Technician Initials
	Positive control[1]	Negative control[2]		
1				
2				
3				
4				
5				
6				
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30				
31				

[1]MTB strain tested/ positive control		[2]Strain tested/negative control	

Reviewed by:

Date**

Signature:

Comments:

*Confirmation required by another technician or prepare another smear, stain and read. **Date format: dd/mmm/yyyy

Appendix C: Ziehl-Neelsen Daily QC Staining Form

Month: ..... Year: .....

Note: If QC is out of range, inform the supervisor and record any action taken below.

ZN Stain Batch or Lot #/ Expiration Date*		Acid Alcohol Decolorizer Batch or Lot #/ Expiration Date*		Methylene Blue Batch or Lot #/ Expiration Date*	
Positive Control Slides date of preparation		Positive control slide date of expiration		Negative control slide date of preparation	Negative control slide date of expiration

Day	Results (Positive, Negative)		QC Pass (Yes, No)	Technician Initials
	Positive control[1]	Negative control[2]		
1				
2				
3				
4				
5				
6				
7				
8				
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30				
31				

[1]MTB strain tested/ positive control		[2]Strain tested/negative control	

Reviewed by:

Date*

Signature:

Comments:

*Date format: dd/mmm/yyyy

APPENDIX D: EQUIPMENT TEMPERATURE RECORD FORMS

Appendix D: Freezer Temperature Record Form						
Equip Number: ……			Location (room # or equivalent): ……			
Temperature Range (circle one): –70°C ±10°C –80°C ±10°C			Thermometer #: ……			
Note: If temperature is out of range, inform the supervisor and record any action taken below.						
Month: ……					Year: ……	
Day	Temp 1	Time Taken/ Initials	Temp 2	Time Taken/ Initials	Action/ Adjustments	Maintenance/ Notes
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
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14						
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30						
31						
Date*						
Reviewed by:						
Signature:						
Comments:						

*Date format: dd/mmm/yyyy

Appendix D: Refrigerator Temperature Record Form						
Equip Number:			Location (room # or equivalent):			
Temperature Range: 2°C-8°C			Thermometer #:			
Note: If temperature is out of range, inform the supervisor and record any action taken below.						
Month:					Year:	
Day	Temp 1	Time Taken/ Initials	Temp 2	Time Taken/ Initials	Action/ Adjustments	Maintenance/ Notes
1						
2						
3						
4						
5						
6						
7						
8						
9						
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27						
28						
29						
30						
31						
Date*						
Reviewed by:						
Signature:						
Comments:						

*Date format: dd/mmm/yyyy

Appendix D: Refrigerator/Freezer Record Form								
Equip Number:				Location (room # or equivalent):				
Refrigerator Temp Range: 2-8°C				Freezer Temp Range: −20°C ± 2°C				
Thermometer #:				Thermometer #:				
Note: If temperature is out of range, inform the supervisor and record any action taken below.								
Month:						Year:		
Day	Refrigerator Temp 1	Freezer Temp 1	Time Taken/ Initials	Refrigerator Temp 2	Freezer Temp 2	Time Taken/ Initials	Action/ Adjustments	Maintenance/ Notes
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								
31								
Date*								
Reviewed by:								
Signature:								
Comments:								

*Date format: dd/mmm/yyyy

Appendix D: Incubator Temperature Record Form						
Equip Number:			Location (room # or equivalent):			
Temperature Range: 37°C ± 1°C			Thermometer #:			
Note: If temperature is out of range, inform the supervisor and record any action taken below.						
Month:					Year:	
Day	Temp 1	Time Taken/ Initials	Temp 2	Time Taken/ Initials	Action/ Adjustments	Maintenance/ Notes
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						
31						
Date*						
Reviewed by:						
Signature:						
Comments:						

*Date format: dd/mmm/yyyy

Appendix D: CO_2 Incubator Record Form									
Equip Number:				Location # (room or equivalent):					
Temperature Range: 37°C ± 1°C				Thermometer #:					
CO_2 Range: 5-10%									
Note: If temperature is out of range, inform the supervisor and record any action taken below.									
Month:								Year:	
Day	Temp 1	CO_2% #1	Time Taken/ Initials	Temp 2	CO_2% #2	Time Taken/ Initials	CO_2 tank pressure	Action/ Adjustments	Maintenance/ Notes
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									
Date*									
Reviewed by:									
Signature:									
Comments:									

*Date format: dd/mmm/yyyy

Appendix D: Refrigerated Centrifuge Temperature Record Form						
Equip Number:			Location (room # or equivalent):			
Temperature Range: 4°C-12°C			Thermometer #:			
Note: If temperature is out of range, inform the supervisor and record any action taken below.						
Month:					Year:	
Day	Temp 1	Time Taken/ Initials	Temp 2	Time Taken/ Initials	Action/ Adjustments	Maintenance/ Notes
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						
31						
Date*						
Reviewed by:						
Signature:						
Comments:						

*Date format: dd/mmm/yyyy

Appendix D: Room Temperature Record Form								
Location (room # or equivalent): ……								
Temperature Range: ** see below ……				Thermometer #: ……				
Note: If temperature is out of range, inform the supervisor and record any action taken below.								
Month: ……						Year: ……		
Day	Temp 1		Time Taken	Temp 2		Time Taken	Action/ Adjustments	Maintenance/ Notes
	Min	Max	Initials	Min	Max	Initials		
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								
31								
Date*								
Reviewed by:								
Signature:								
Comments:								

*Date format: dd/mmm/yyyy
**Based on requirements of reagents or instrument housed in the particular room; e.g., MGIT room: 19-30°C; room containing unrefrigerated centrifuge: ≤20°C.

APPENDIX E: NEW REAGENTS/MEDIA QC FORMS

Appendix E: New Reagents/Media QC Form: Acridine Orange							
Lot/Batch Number of Prepared Stain	Date Reagent Prepared*	Expiry Date of Batch*	Quantity Prepared	Date Tested*/ Tech Initials	Results (Neg, Confirmation Required**, Scanty, 1+, 2+, 3+)		QC pass? (Yes/No)
					Positive Control[1]	Negative Control[2]	
[1]MTB strain tested/ positive control		[2]Strain tested/ negative control			Monthly Supervisor Review (date*/initials)		

New Lot/Batch Staining Reagents						
Component	Brand (Manufacturer)	Lot Number	Received Date*	Expiry Date*	Quantity Received	Date Opened*
Acridine Orange						
Sodium carbonate						
Sulfuric acid						
Benzalkonium chloride						
Sodium barbital/Tricine						
Ethanol						
HCL, concentrated						

*Date format = dd/mmm/yyyy, **Confirmation required by another technician or prepare another smear, stain and read.

Appendix E: New Reagents/Media QC Form: Auramine O							
Lot/Batch Number of Prepared Stain	Date Reagent Prepared*	Expiry Date of Batch*	Quantity Prepared	Date Tested*/ Tech Initials	Results (Neg, Confirmation Required**, Scanty, 1+, 2+, 3+)		QC pass? (Yes/No)
					Positive Control[1]	Negative Control[2]	
[1]MTB strain tested/ positive control		[2]Strain tested/ negative control			Monthly Supervisor Review (date*/initials)		

New Lot/Batch Staining Reagents						
Component	Brand (Manufacturer)	Lot Number	Received Date*	Expiry Date*	Quantity Received	Date Opened*
Auramine O						
Phenol						
Ethanol						
HCL, concentrated						
Potassium permanganate						

*Date format = dd/mmm/yyyy, **Confirmation required by another technician or prepare another smear, stain and read.

Appendix E: New Reagents/Media QC Form: Auramine O (Commercial)														
Auramine O				Acid Alcohol				Potassium Permanganate				Results (Neg, Confirmation Required**, Scanty, 1+, 2+, 3+)		QC Pass (Yes, No)/ Tech Initials
Brand/Lot Number	Rec'd Date*/ Expiration Date*	Quantity Rec'd	Date* Tested	Brand/Lot Number	Rec'd Date*/ Expiration Date*	Quantity Rec'd	Date* Tested	Brand/Lot Number	Rec'd Date*/ Expiration Date*	Quantity Rec'd	Date* Tested	Positive control[1]	Negative control[2]	
[1]MTB strain tested/ positive control				[2]Strain tested/negative control				Monthly Supervisor Review (date*/initials)						

*Date format = dd/mmm/yyyy, **Confirmation required by another technician or prepare another smear, stain and read.

Appendix E: New Reagents/Media QC Form: Auramine/Rhodamine							
Lot/Batch Number of Prepared Stain	Date Reagent Prepared*	Expiry Date of Batch*	Quantity Prepared	Date Tested*/ Tech Initials	Results (Neg, Confirmation Required**, Scanty, 1+, 2+, 3+)		QC pass? (Yes/No)
					Positive Control[1]	Negative Control[2]	
[1]MTB strain tested/ positive control		[2]Strain tested/ negative control			Monthly Supervisor Review (date*/initials)		

New Lot/Batch Staining Reagents						
Component	Brand (Manufacturer)	Lot Number	Received Date*	Expiry Date*	Quantity Received	Date Opened*
Auramine O						
Phenol						
Ethanol						
HCL, concentrated						
Potassium permanganate						

*Date format = dd/mmm/yyyy, **Confirmation required by another technician or prepare another smear, stain and read.

Appendix E: New Reagents/Media QC Form: Auramine/Rhodamine (Commercial)															
Auramine/Rhodamine				Acid Alcohol				Potassium Permanganate				Results (Neg, Confirmation Required**, Scanty, 1+, 2+, 3+)		QC Pass (Yes, No)/ Tech Initials
Brand/Lot Number	Date*/ Expiration Date*	Quantity Rec'd	Date* Tested	Brand/Lot Number	Date*/ Expiration Date*	Quantity Rec'd	Date* Tested	Brand/Lot Number	Date*/ Expiration Date*	Quantity Rec'd	Date* Tested	Positive control[1]	Negative control[2]	
[1]MTB strain tested/ positive control				[2]Strain tested/negative control				Monthly Supervisor Review (date*/initials)						

*Date format = dd/mmm/yyyy, **Confirmation required by another technician or prepare another smear, stain and read.

Appendix E: New Reagents/Media QC Form: Ziehl-Neelsen							
Lot/Batch Number of Prepared Stain	Date Reagent Prepared*	Expiry Date of Batch*	Quantity Prepared	Date Tested*/ Tech Initials	Results (Positive, Negative)		QC pass? (Yes/No)
					Positive Control[1]	Negative Control[2]	
[1]MTB strain tested/ positive control		[2]Strain tested/ negative control			Monthly Supervisor Review (date*/initials)		

New Lot/Batch Staining Reagents						
Component	Brand (Manufacturer)	Lot Number	Received Date*	Expiry Date*	Quantity Received	Date Opened*
Fuchsin						
Phenol						
Ethanol						
HCL, concentrated						
Methylene blue						

*Date format = dd/mmm/yyyy,

Appendix E: New Reagents/Media QC Form: Ziehl-Neelsen (Commercial)														
Carbol Fuchsin Reagent				Acid Alcohol				Methylene Blue				Results (Positive, Negative)		QC Pass (Yes, No)/ Tech Initials
Brand/Lot Number	Date*/ Expiration Date*	Quantity Rec'd	Date* Tested	Brand/Lot Number	Date*/ Expiration Date*	Quantity Rec'd	Date* Tested	Brand/Lot Number	Date*/ Expiration Date*	Quantity Rec'd	Date* Tested	Positive control[1]	Negative control[2]	
[1]MTB strain tested/ positive control				[2]Strain tested/negative control				Monthly Supervisor Review (date*/initials)						

*Date format = dd/mmm/yyyy,

Appendix E: New Reagents/Media QC Form: Processing Reagents							
Reagent Name	Lot/batch Number of Prepared Reagent	Date Reagent Prepared*	Expiry Date*	Quantity Prepared	pH (for Phosphate Buffer only)	Date Put into Use*	Tech Initials
					Monthly Supervisor Review (date*/ initials)		

New Lot/Batch Reagents						
Component	Brand (Manufacturer)	Lot Number	Received Date*	Expiry Date*	Quantity Received	Date Opened*
NALC						
Sodium hydroxide						
Sodium citrate						
Disodium phosphate						
Monopotassium phosphate						

*Date format = dd/mmm/yyyy,

Appendix E: New Reagents/Media QC Form: MGIT							
Lot Number	MGIT Supplement Number	Date Tested*	Tech Initials	Results MTB 6-10 days[1]	Results: Other Strains[2]	Date Result*/ Tech Initials	QC pass? (Yes/No)
[1]MTB strain tested		[2]Other strains (if tested)			Monthly Supervisor Review (date*/ initials)		

New Lot/Batch Media					
Component/Brand (Manufacturer)	Lot Number	Received Date*	Expiry Date*	Quantity Received	Date Put in Use*
BD - MGIT 7 ml tubes					
BD - MGIT 7 ml tubes					
BD - MGIT 7 ml tubes					

*Date format = dd/mmm/yyyy,

Appendix E: New Reagents/Media QC Form: MGIT Supplement						
Supplement Lot Number	MGIT Media Lot Number	Date Tested*/ Tech Initials	Results MTB 6-10 days[1]	Results: Other Strains[2]	Date Result*/ Tech Initials	QC pass? (Yes/No)
[1]MTB strain tested		[2]Other strains (if tested)		Monthly Supervisor Review (date*/ initials)		

New Lot/Batch Reagents					
Component	Lot Number	Received Date*	Expiration Date*	Quantity Received	Date Put in Use*
BD MGIT Supplement					
BD MGIT PANTA					
BD MGIT Supplement					
BD MGIT PANTA					
BD MGIT Supplement					
BD MGIT PANTA					

*Date format = dd/mmm/yyyy,

Appendix E: New Reagents/Media QC Form: Lowenstein Media									
Lot/Batch Number of Prepared/ Media	Date Media Prepared*/ Tech Initials	Expiry Date of Batch*	Quantity Media Prepared	Date Tested*/Tech Initials	Results: MTB growth[1]			Results Sterility Control[2]	QC pass? (Yes/No)/ Date*/Tech Initials
					10^{-2}	10^{-3}	10^{-4}		
[1]MTB strain tested/ positive control		[2]Unino-culated media			Monthly Supervisor Review (date*/initials)				

New Lot/Batch Media						
Component	Brand (Manufacturer)	Lot Number	Received Date*	Expiry Date*	Quantity Received	Date Put in Use*
L-J Media Base						
Glycerol						
KH_4PO_4						
Sodium glutamate						
Malachite green						

*Date format = dd/mmm/yyyy,

Appendix E: New Reagents/Media QC Form: Lowenstein Jensen Media (Commercial)									
Brand (Manufacturer)	Lot Number	Received Date*	Expiration Date*	Quantity Received	Appearance		Date* Put into Use	QC pass? (Yes/No)	Tech Initials/ Date*
					Acceptable	Not Acceptable **			
Comments/ corrective actions:						Monthly Supervisor Review (date*/initials)			

*Date format = dd/mmm/yyyy, ** Explain in comments section

Appendix E: New Reagents/Media QC Form: 7H9 Broth									
Lot/Batch Number Prepared Broth	Date Broth Prepared*/ Tech Initials	Expiry Date of Batch*	Quantity Prepared	Date Tested*/ Tech Initials	Results: Days MTB growth[1]		Results Sterility Control[2]	Date*/ Tech Initials	QC pass? (Yes/No)
					10^{-2}	10^{-4}			
[1]MTB strain tested		[2]Unino-culated tubes			Monthly Supervisor Review (date*/initials)				

New Lot/Batch Media						
Component	Brand (Manufacturer)	Lot Number	Received Date*	Expiry Date*	Quantity Received	Date Opened*
Middlebrook 7H9 basal medium						
Glycerol						
ADC or OADC supplement						

*Date format = dd/mmm/yyyy,

Appendix E: New Reagents/Media QC Form: BAP									
Lot/Batch Number Prepared Media	Blood Source Number	Date Media Prepared*/Tech Initials	Expiry Date of Batch*	Quantity Prepared	Date Tested*/ Tech Initials	Results: Positive Growth[1]	Results: Sterility Control[2]	Date*/ Tech Initials	QC pass? (Yes/No)
[1]Strain tested for growth performance		[2]Unino-culated plates			Monthly Supervisor Review (Initials/Date*)				

New Lot/Batch Media						
Component	Brand (Manufacturer)	Lot Number	Received Date*	Expiry Date*	Quantity Received	Date Opened*
TSA base						

*Date format = dd/mmm/yyyy,

Appendix E: New Reagents/Media QC Form: Bloor Agar Media (Commercial)									
Brand (Manufacturer)	Lot Number	Received Date*	Expiration Date*	Quantity Received	Appearance		Date* Put into Use	Date/Tech Initials	QC pass? (Yes/No)
					Acceptable	Not Acceptable **			
Comments/ corrective actions:						Monthly Supervisor Review (date*/initials)			

*Date format = dd/mmm/yyyy, ** Explain in comments section

Appendix E: New Reagents/Media QC Form: TBc ID Extraction Buffer								
Lot/Batch Number of Prepared Buffer	Date Buffer Prepared*/ Tech Initials	Expiry Date of Batch*	Quantity Prepared	Date Tested*/ Tech Initials	Lot Number TBc ID Kit	Results (Positive, Negative)		QC pass? (Yes/No)
						Positive Control[1]	Negative Control[2]	
[1]MTB strain tested		[2]Strain tested/ negative control			Monthly Supervisor Review (date*/initials)			

New Lot/Batch Extraction Buffer						
Component	Brand (Manufacturer)	Lot Number	Received Date*	Expiry Date*	Quantity Received	Date Opened*
Monopotassium phosphate						
Sodium chloride						
Tween 80						

*Date format = dd/mmm/yyyy,

APPENDIX F: DST QC/INVENTORY FORMS

Appendix F: DST QC Form - BD MGIT Drugs								
Drug Tested (SIRE, PZA)	MGIT Media Lot Number	SIRE or PZA Supplement Number	Date Tested*/ Tech initials	Results: MTB[1]		Results: Other Strains[2]		QC pass? (Yes/No) Tech initials/ Date*
				TTD (days, hours)	Drug result (S/R)	TTD (days, hours)	Drug result (S/R)	
[1]MTB strain tested			[2]Other strains (if tested)			Supervisor Review (date*/initials)		

Drug Information					
Manufacturer/ Drug	Drug Lot Number	Received Date*	Expiration Date*	Quantity Received	Date Opened*
BD - Streptomycin					
BD - Isoniazid					
BD - Rifampicin					
BD - Ethambutol					
BD - PZA					

*Date format = dd/mmm/yyyy,

Appendix F: DST QC Form - MGIT Second Line Drugs								
Drug Tested	MGIT Media Lot Number	SIRE Supplement Number	Date Tested*/ Tech initials	Results: MTB[1]		Results: Other Strains[2]		QC pass? (Yes/No) Tech initials/ Date*
				TTD (days, hours)	Drug result (S/R)	TTD (days, hours)	Drug result (S/R)	
[1]MTB strain tested			[2]Other strains (if tested)			Supervisor Review (date*/initials)		

Drug Information							
Drug	Amikacin	Capreomycin	Gatifloxacin	Kanamycin	Levofloxacin	Moxifloxacin	Ofloxacin
Source (Brand)							
Drug Lot Number							
Received Date*							
Expiration Date*							
Quantity							
Date Opened*							

*Date format = dd/mmm/yyyy,

Appendix F: DST QC Form - Drugs Tested on Solid Media										
Drug	Drug Source	Drug Lot Number	Date of Manufacture*	Expiry Date*	Date Drug Opened*	Date Drug Stock Solution Made*	Results MTB strain[1] (S/R)	Results: Other strain[2] (S/R)	Media Lot Number	QC pass? (Yes/No)/ Tech initials/Date*
[1]MTB strain tested			[2]Other strains (if tested)			Date tested*/ Tech Initials:			Supervisor Review (date*/initials)	

*Date format = dd/mmm/yyyy,

Appendix F: MGIT Second-Line Drugs - Inventory Worksheet								
Drug Name			Drug Name			Drug Name		
Purity (Potency)			Purity (Potency)			Purity (Potency)		
Drug Lot Number			Drug Lot Number			Drug Lot Number		
Date*	Amount Used	Amount Remaining	Date*	Amount Used	Amount Remaining	Date*	Amount Used	Amount Remaining

*Date format = dd/mmm/yyyy,

APPENDIX G

Appendix G: MTB Identification QC Form - ICA Slide Test MPT64/MTB 64						
Lot Number	Date Tested*/ Tech Initials	Results: Internal Control (Positive, Negative)	Results: Positive Control[1] (Positive, Negative)	Results: Negative Control[2] (Positive, Negative)	QC Pass? (Yes/No)	Date*/ Tech Initials
[1]MTB strain tested/positive control:		[2]Strain tested/ negative control:		Supervisor Review (Date*/Initials)		

Brand (Manufacturer)	Lot Number	Received Date*	Expiry Date*	Quantity Received	Date Opened*

*Date format = dd/mmm/yyyy,

APPENDIX H

Appendix H: Weekly MGIT/LJ Culture QC Form

Specimen Type (Pos/Neg)	Date Tested*/ Tech initials	Lab Accession Number	MGIT or LJ Media Lot Number	Expiry Date*	Smear Result (Neg, Confirmation required, Scanty, 1+ 2+ 3+)	Results: [1,2]			QC pass? (Yes/No)/ Tech initials/ Date	Comments
						Growth (Pos, Neg)	MGIT: TTD (days, hours)	LJ: number of colonies		
Positive										
Negative										
Positive										
Negative										
Positive										
Negative										
Positive										
Negative										
Positive										
Negative										
Positive										
Negative										
Positive										
Negative										
Positive										
Negative										
Positive										
Negative										
Positive										
Negative										
Positive										
Negative										
[1]MTB strain tested			[2]Negative control (neg sputum, 7H9 broth, etc.)				Supervisor Review (Date*/Initials)			

*Date format = dd/mmm/yyyy,

APPENDIX I: MONTHLY DATA MONITORING FORMS

Appendix I: MONTHLY DATA MONITORS - AFB MICROSCOPY														
Year:										Supervisor Review (Date (dd/mmm/yyyy)/initials):				
	SPECIMEN TALLY*									AFB MICROSCOPY*				
	Received			Unacceptable			Completed			Total Smears Read	Smear Positive		Smear Negative	
Month/ Tech Initials	Pt. collected	Site collected	Total	Pt. collected	Site collected	Total	Pt. collected	Site collected	Total		Total	Percent	Total	Percent
Jan														
Feb														
March														
April														
May														
June														
July														
Aug														
Sept														
Oct														
Nov														
Dec														

*Calculate numbers based upon cultures **received** in the month.

Appendix I: MONTHLY DATA MONITORS - MGIT CULTURES																
Year:											Supervisor Review (Date (dd/mmm/yyyy)/initials):					
				Positive Cultures*		Culture Positive/ Smear Positive*			Culture Positive/Smear Negative*			Negative Cultures*		Culture Negative/ Smear Positive*		Culture Negative/ Smear Negative*	
Month/ Tech Initials	Total Reported*			Total	Percent	Total	Percent	Average TTD (Days)	Total	Percent	Average TTD (Days)	Total	Percent	Total	Percent	Total	Percent
	Pt. collected	Site collected	Total														
Jan																	
Feb																	
March																	
April																	
May																	
June																	
July																	
Aug																	
Sept																	
Oct																	
Nov																	
Dec																	

*Calculate numbers based upon cultures **reported** in the month.

Appendix I: MONTHLY DATA MONITORS - LJ CULTURES																	
Year:														Supervisor Review (Date (dd/mmm/yyyy)/initials):			
				Positive Cultures*		Culture Positive/ Smear Positive*			Culture Positive/Smear Negative*			Negative Cultures*		Culture Negative/ Smear Positive*		Culture Negative/ Smear Negative*	
Month/ Tech Initials	Total Reported*			Total	Percent	Total	Percent	Average TTD (Weeks)	Total	Percent	Average TTD (Weeks)	Total	Percent	Total	Percent	Total	Percent
	Pt. collected	Site collected	Total														
Jan																	
Feb																	
March																	
April																	
May																	
June																	
July																	
Aug																	
Sept																	
Oct																	
Nov																	
Dec																	

*Calculate numbers based upon cultures **reported** in the month.

Appendix I: MONTHLY DATA MONITORS - CONTAMINATION RATES

Year: ..... Supervisor Review (Date (dd/mmm/yyyy)/initials): .....

Month/ Tech Initials	MONTHLY MGIT CULTURES*										MONTHLY LJ CULTURES*									
	Number MGIT Cultures Reported*			Number of Contaminated MGIT Cultures*			Contamination Rate (# Contaminated/ # Reported)*			Cumulative MGIT Contamination Rate (Total %)	Number LJ Cultures Reported*			Number of Contaminated LJ Cultures*			Contamination Rate for LJ Cultures (# Contaminated/ # Reported)*			Cumulative LJ Contamination Rate (Total %)
	Pt. coll.	Site coll.	Total	Pt. coll.	Site coll.	Total	Pt. coll.	Site coll.	Total		Pt. coll.	Site coll.	Total	Pt. coll.	Site coll.	Total	Pt. coll.	Site coll.	Total	
Jan																				
Feb																				
March																				
April																				
May																				
June																				
July																				
Aug																				
Sept																				
Oct																				
Nov																				
Dec																				

*Calculate numbers based upon cultures **reported** in the month.
Pt. coll = Patient-collected specimens Site coll = site-collected specimens

Appendix I: MONTHLY DATA MONITORS - ISOLATION RATES MTB and MOTT

Year: ..... Supervisor Review (Date (dd/mmm/yyyy)/initials): .....

	Isolation of Mycobacterium tuberculosis Complex*								Isolation of MOTT*							
	MGIT				LJ				MGIT				LJ			
Month/ Tech Initials	Total # MGIT cultures reported	Total # MTB pos MGIT cultures	Percent specimens yielding MTB	Average MTB isolation rate (%)	Total # LJ cultures reported	Total # MTB pos LJ cultures	Percent specimens yielding MTB	Average MTB isolation rate (%)	Total # MGIT cultures reported	Total # MOTT pos MGIT cultures	Percent specimens yielding MOTT	Average MOTT isolation rate (%)	Total # LJ cultures reported	Total # MOTT pos LJ cultures	Percent specimens yielding MOTT	Average MOTT isolation rate (%)
Jan																
Feb																
March																
April																
May																
June																
July																
Aug																
Sept																
Oct																
Nov																
Dec																

*Calculate numbers based upon cultures **reported** in the month.

APPENDIX J: STORAGE LOG FORMS

Appendix J: Freezer Storage Log for *M. tuberculosis* Isolates											
Freezer #:					Location (room # or equivalent):						
Rack Number	Box Number	Position # in Box	Screening ID Number	Subject ID Number	Visit Number	Lab Accession Number	Sputum Specimen Number*	Aliquot #*	Date of Collection **	Date Frozen**/ Tech Initials	Date shipped** (if applicable)
								Supervisor Review (Date**/Initials)			

* Sputum specimen number should be #1 or #2 (or N/A if from visits 2 or 3). Aliquot # should be 1-4 (or N/A if from visits other than 2 or 3).
** Date format: dd/mmm/yyyy

Appendix J: Storage Log for *M. tuberculosis* Isolates on LJ Media										
Rack Number (if applicable)	Box Number	Position # in Box	Screening ID Number	Subject ID Number	Visit Number	Lab Accession Number	Sputum Specimen Number*	Date of Collection **	Date Stored**/ Tech Initials	Date Discarded**/ Tech Initials
								Supervisor Review (Date**/Initials)		

* Sputum specimen number should be #1 or #2 (or N/A if from visits 2 or 3).

** Date format: dd/mmm/yyyy

APPENDIX K: CONTINUOUS QUALITY IMPROVEMENT FORM

QI Number: ..... Date* Form Prepared: .....

Type of Issue: ☐ Clerical error ☐ Equipment problem ☐ Procedural error
☐ QC failure ☐ Supply problem ☐ Other (list): .....

SECTION I: SUMMARY OF ISSUE

Date* of occurrence: .....

Describe the problem, issue, preventable error, etc.:

.....

.....

Has this same issue occurred previously? ☐ Yes ☐ No ☐ Unknown

If Yes, include QI form number: .....

SECTION II: INVESTIGATION

Describe the root cause of the problem:

.....

SECTION III: CORRECTIVE ACTION FOR THIS INCIDENT:

Describe corrective action taken to resolve this issue:

.....

.....

Expected date* for resolution: .....

Did this incident impact any patient results? ☐ Yes ☐ No ☐ N/A

If Yes, describe:

.....

.....

Was retesting necessary? ☐ Yes ☐ No ☐ N/A

If Yes, date* of re-test: ..... Results acceptable? ☐ Yes ☐ No

Comments:

.....

.....

Attach documentation to this report

SECTION IV: PREVENTION

Describe policies, practices, etc. to be implemented as a result of this investigation to prevent further occurrences (if applicable):

.....

.....

Identify the individual(s) responsible for monitoring the effectiveness of these policies/practices:

.....

Date:*..... Name/Signature/Title of Person Identifying Issue.....

Date:*..... Name/Signature/Title of Responsible Person.....

Date:*..... Name/Signature/Title of Supervisor.....

* Date format: dd/mmm/yyyy

APPENDIX L: MGIT TTD WORKSHEET

MTB strain used for dilutions:		Source of strain (ATCC, WHO, etc.):	
# of subcultures (transfers from original stock):		Is this strain pan-susceptible? (Yes/No)	
Type of suspension used (frozen or fresh subculture; MGIT):			

TTD Results

	TTD (days and hours)	Tech Initials	Supervisor Review (Date**/Initials)
Date Inoculated**	1:500 (Tube 3*)		

* Estimated Concentrations: Tube 3 - log 10^5
** Date format: dd/mmm/yyyy

APPENDIX M: EARLY MGIT POSITIVES/ EARLY CONTAMINATED CULTURES TRACKING WORKSHEET

Screening/Subject ID		Visit #		Sputum Specimen #		Lab Accession #	
Date* of Positivity:		TTD		ZN Result:		BAP Result:	
Reincubated:	☐ Yes ☐ No	Date:		Number of Days:		Location:	☐ MGIT ☐ Off-line
Results for 1st Reincubation							
Date* of Positivity:		TTD from MGIT printout (if applicable):		ZN Result:		BAP Result:	
Reincubated:	☐ Yes ☐ No	Date*:		Number of Days:		Location:	☐ MGIT ☐ Off-line
Results for 2nd Reincubation							
Date* of Positivity:		TTD from MGIT printout (if applicable):		ZN Result:		BAP Result:	
Reincubated:	☐ Yes ☐ No	Date*:		Number of Days:		Location:	☐ MGIT ☐ Off-line
Results for 3rd Reincubation							
Date* of Positivity		TTD from MGIT printout (if applicable):		ZN Result:		BAP Result:	
Reincubated:	☐ Yes ☐ No	Date*:		Number of Days:		Location:	☐ MGIT ☐ Off-line
End of 42 Day Protocol							
Date of MGIT result at 42 days:		TTD		ZN Result:		BAP Result:	
Tube Turbid?	☐ Yes ☐ No	ID Result:	☐ Pos MTB ☐ Neg MTB	LJ Subculture Date*:		Sponsor Notified (Date*/Tech):	

Final Results[1]

Final Date of MGIT result:		Final ID result:	☐ Pos MTB ☐ Neg MTB ☐ N/A	Final TTD:		Final Culture Result:	☐ Negative MTB ☐ Positive MTB ☐ Positive MTB and contam ☐ No TB growth, positive MOTT ☐ Contam ☐ Unknown	Supervisor Review (Initials/ Date*):	
COMMENTS:									

*Date format = dd/mmm/yyyy TTD = time to detection ZN = Ziehl-Neelsen BAP = Blood Agar Plate Contam = contaminated

[1] Final results section to be completed after the reincubation step that provides sufficient data to result the culture.

APPENDIX N: LABORATORY VISITOR LOG

Page [ ] of [ ]

Protocol No.: ..... Lab Name: .....

Lab Director Name: .....

Address / Location: .....

.....

Date* of Visit (Record each day of visit on a separate line)	Printed Name of Visitor	Title of Visitor	Purpose of Visit	Signature of Visitor	Printed Name of Laboratory Personnel	Initials

** Date format: dd/mmm/yyyy