Centre for Clinical Microbiology

# Overview of Mycobacterium Growth Indicator Tubes (MGIT) including Drug Susceptibility Testing (DST)

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# MGIT Overview

1. Principles
2. Reagents
3. Inoculation
4. Unloading MGIT tubes
5. Processing MGIT tubes
6. Reporting results

# 1. Principles


- Fluorescent compound present in the silicone at the bottom of MGIT
- Fluorescent compound is sensitive to the presence of oxygen
- Initially there is large amounts of dissolved oxygen
- The dissolved oxygen decreases as the *Mycobacterium spp* grow
- This results in increased fluorescence
- MGIT incubation is at 35-37°C
- Each MGIT is monitored every 60 minutes for increasing fluorescence

# 1. Principles

Initially

↑ Dissolved Oxygen

↓ Little Fluorescence

+

↓ Dissolved Oxygen

↑ Increased Fluorescence

# 1. Principles

- A **positive** MGIT contains $10^5$ to $10^6$ colony forming units per milliliter (CFU/mL)
- A **negative** MGIT has no fluorescence/visible signs of bacterial growth at 42 days

# 2. Reagents

MGIT tubes contain:

- ✓ 110 µL fluorescent indicator
- ✓ 7 mL modified Middlebrook 7H9 broth

# 2. Reagents

- Growth supplement (OADC) is added to tubes to enable the rapid growth of mycobacteria

| Middlebrook OADC supplement – values per L Purified Water | | |
|---|---|---|
| **O**leic Acid | 0.1 g | Involved in metabolism of mycobacteria |
| **A**lbumin | 50.0 g | Protective agent by binding free fatty acids which may be toxic to Mycobacterium species |
| **D**extrose | 20.0 g | Energy Source |
| **C**atalase | 0.03 g | Destroys toxic peroxides that may be present in media |
| Polyoxyethylene stearate (POES) | 1.1 g | Emulsifying agent. Encourages Mycobacterium growth |

# 2. Reagents

- Contamination is reduced when PANTA antibiotic mixture is added to the media

| PANTA: per vial of lyophilised PANTA – per L purified water | |
|---|---|
| Polymyxin B | 6,000 units |
| Amphotericin B | 600 µg |
| Nalidixic acid | 2,400 µg |
| Trimethoprim | 600 µg |
| Azlocilin | 600 µg |

# 3. Inoculation

1. Reconstitute PANTA powder with growth supplement (15mL)
2. Label the MGIT with specimen number & date
3. Add 800 µL of growth supplement/PANTA to MGIT tube
4. Add 500 µL of concentrated specimen suspension as prepared
5. Tightly recap the tube and invert gently
6. Scan in the tube (BD BACTEC MGIT machine) – tubes automatically tested for the recommended 42 day testing protocol

# 3. Inoculation

All sub-culturing must take place using **bio-safety level III** practices and containment facilities

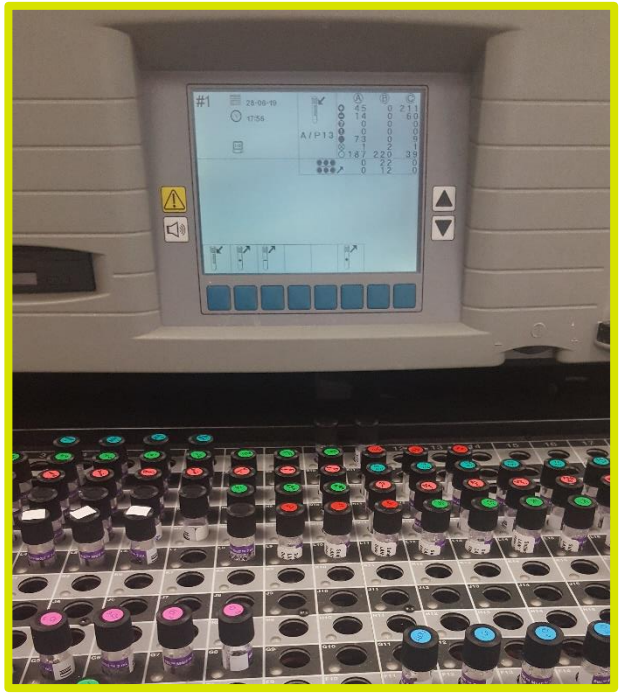
Exact practices may vary between sites due to individual requirements. This will be highlighted with a star (*)

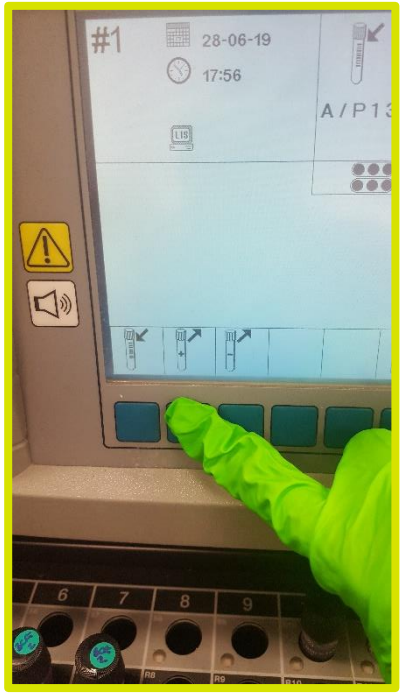
# 4. Unloading MGIT tubes

- After pressing the unload positive button
- Any positive MGIT will show a flashing light in the position it has been place in the machine
- The tube will need to be scanned out
- A positive report can be printed and annotated as required

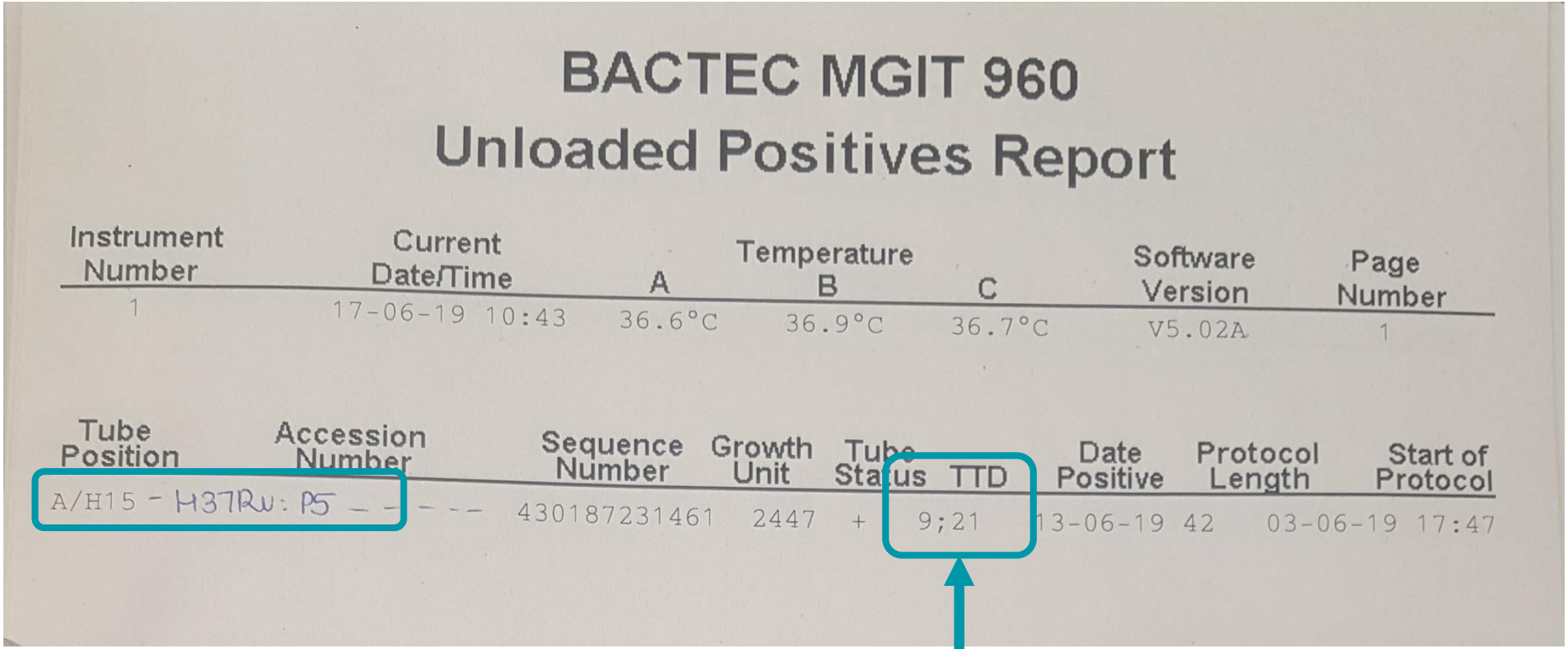
- Negative MGIT tubes are unloaded similarly, after pressing the unload negative button

# 4. Unloading MGIT tubes






# 4. Unloading MGIT tubes



BACTEC MGIT 960

Unloaded Positives Report

| Instrument Number | Current Date/Time | Temperature A | Temperature B | Temperature C | Software Version | Page Number |
|---|---|---|---|---|---|---|
| 1 | 17-06-19 10:43 | 36.6°C | 36.9°C | 36.7°C | V5.02A | 1 |

| Tube Position | Accession Number | Sequence Number | Growth Unit | Tube Status | TTD | Date Positive | Protocol Length | Start of Protocol |
|---|---|---|---|---|---|---|---|---|
| A/H15 - H37Rv: P5 | - - - - | 430187231461 | 2447 | + | 9;21 | 13-06-19 | 42 | 03-06-19 17:47 |

Must be ≥ 4 days

# 5. Processing tubes: Positive

1. Scan out a tube
2. Positive tubes for *Mycobacterium tuberculosis* should flag on or after 4 days
3. Positive tubes should be sub-cultured onto solid-LJ/liquid media-MGIT as required*
4. An acid-fast smear should be prepared
5. Using a sterile pipette remove approximately 100 µL from the bottom of the tube for stain preparations
6. Report preliminary results only after acid-fast smear evaluation

# 5. Processing tubes: Negative

1. Scan out a tube

2. Negative tubes should be inspected after the 42 day period for positivity

3. If any signs of positivity, it should be subcultured and treated as a presumptive positive, provided the acid-fast smear result is positive

4. If there is no sign of positivity, the tube should be removed from the MGIT instrument and sterilised before discarding

# 5. Reporting Results

- Results should be reported as per the requirements of the individual institute*

- This may vary between sites*

# Drug Susceptibility Testing

1. Overview
2. MDR / XDR TB
3. Principle
4. Preparation of drugs
5. Preparation of MGIT
6. Using inoculum from a positive MGIT
7. Growth control preparation
8. Inoculation of MGIT
9. Loading DST
10. Interpreting DST results
11. Confirming resistant isolates
12. Error messages
13. Quality Control

# Drug Susceptibility Testing

Detection of resistance in *Mycobacterium tuberculosis* is of great importance for:

Effective Patient Management

Infection Control

# Drug Susceptibility Testing

- Drug susceptibility testing is a rapid qualitative procedure for susceptibility testing of *Mycobacterium tuberculosis*

- Treatment of TB is most commonly through a multiple drug regimen that includes: Streptomycin (STR), Isoniazid (INH), Rifampicin (RIF), Ethambutol (EMB) and Pyrazinamide (PZA)

- It is imperative the drugs prescribed for any particular patient show appropriate activity against *Mycobacterium tuberculosis*

# Drug Susceptibility Testing: MDR-TB

- Resistance to the first line drugs Isoniazid and Rifampicin is increasing globally
- Resistance to these drugs defines multi drug resistant tuberculosis (MDR TB)
- MDR TB is linked to significant mortality and is a serious threat to the efficacy of TB control programs

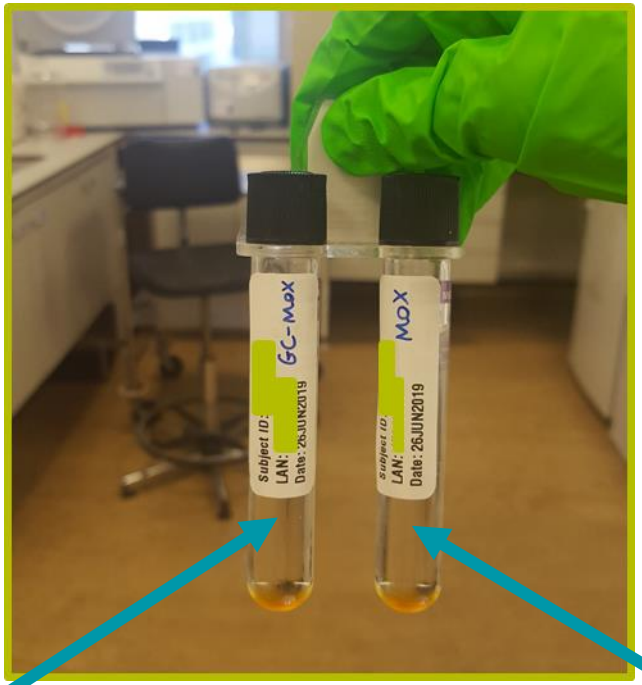
# Drug Susceptibility Testing: XDR-TB

Extensively drug resistant tuberculosis (XDR-TB) is defined as:

- MDR-TB with resistance to second line fluoroquinolones (*e.g.* moxifloxacin)
- Plus resistance to one of the second line aminoglycosides (*e.g.* kanamycin)
- XDR-TB poses a greater challenge to treatment in patients

# Drug Susceptibility Testing Principle

The BD BACTEC MGIT 960 DST principle is based on growth of the *Mycobacterium tuberculosis* isolate in a drug containing tube compared to a drug free tube (Growth Control-GC)

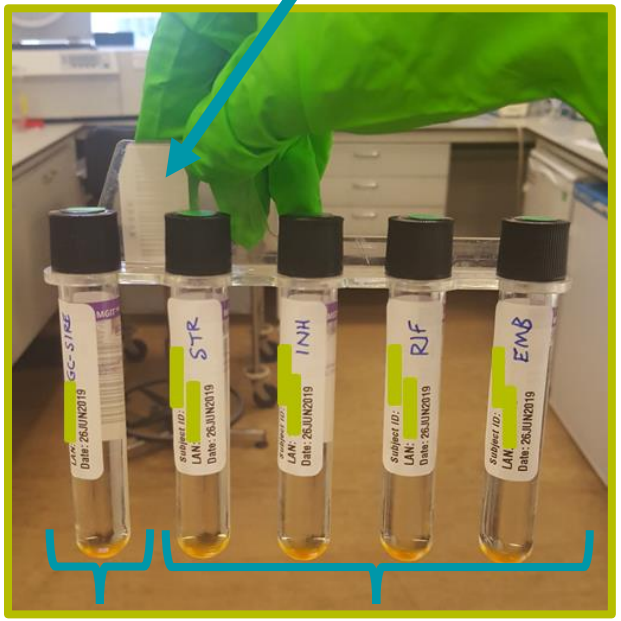



Growth Control

Drug containing tube

# Drug Susceptibility Testing Principle Overview

- DST is performed using an AST (antibiotic susceptibility testing) set
- The set consists of a **drug-free growth control MGIT tube (GC)** and **one MGIT 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
- Growth of the specimen added to the drug containing tube is compared with the drug-free growth control which contains the same specimen
- The bacterial inoculum added to the drug-free growth control tube is one hundred-fold less than the inoculum added to the drug containing tubes



# Preparation of drugs

## First line drugs:

| Drug | Stock concentration of drug* | Volume added to MGIT tubes for test | Final critical concentration in MGIT tubes | Supplier (Becton Dickinson - BD) |
|---|---|---|---|---|
| **Streptomycin (STR)** | 83 µg/ml | 100 µl | 1.0 µg/ml | BD (SIRE kit) |
| **Isonazid (INH)** | 8.3 µg/ml | 100 µl | 0.1 µg/ml | BD (SIRE kit) |
| **Rifampicin (RIF)** | 83 µg/ml | 100 µl | 1.0 µg/ml | BD (SIRE kit) |
| **Ethambutol (EMB)** | 415 µg/ml | 100 µl | 5.0 µg/ml | BD (SIRE kit) |

* These drugs must be reconstituted using 4 mL of sterile/deionised water to achieve concentrations indicated in the table above.

| Drug | Stock concentration of drug* | Volume added to MGIT tubes for test | Final critical concentration in MGIT tubes | Supplier (Becton Dickinson - BD) |
|---|---|---|---|---|
| **Pyrazinamide** | 8000 µg/ml | 100 µl | 100 µg/ml | BD (PZA kit) |

* PZA must be reconstituted using 2.5 mL of sterile/deionised water to achieve concentrations indicated in the table above.

# Preparation of drugs

## Second line drugs:

| Drug | Stock concentration of drug* | Volume added to MGIT tubes for test | Final critical concentration in MGIT tubes | Supplier (Becton Dickinson - BD) |
|---|---|---|---|---|
| Moxifloxacin (MOX) | 20.75 µg/ml | 100 µl | 0.25 µg/ml | BD (Moxifloxacin HCl) |
| Kanamycin (KAN) | 207.5 µg/ml | 100 µl | 2.5 µg/ml | BD (Kanamycin Sulphate) |

* MOX must be reconstituted using 3 mL of sterile/deionised water and then diluted 1:8 with sterile/deionised water to achieve concentrations indicated in the table above.

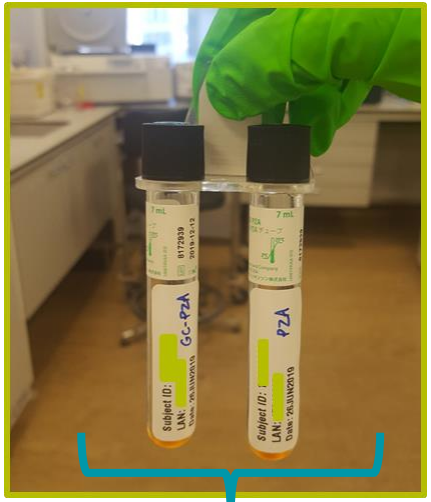
* KAN must be reconstituted using 4 mL of sterile/deionised water to achieve concentrations indicated in the table above.

# Pyrazinamide (PZA) Differences

- The activity of PZA requires a lower pH of 6.0
- Green tubes provided by BD are used for PZA DST testing
- The growth control for PZA is also incubated in a green tube as the lower pH of 6.0 does not inhibit growth of *Mycobacteria tuberculosis*

# Preparation of MGIT tubes for DST testing: CL2

1. Label as many tubes as required for testing
2. Place tubes in correct sequence
3. Aseptically add 800 µL of BACTEC MGIT Supplement (SIRE and/or PZA)
4. Aseptically pipette 100 µL of drug




800 µL supplement

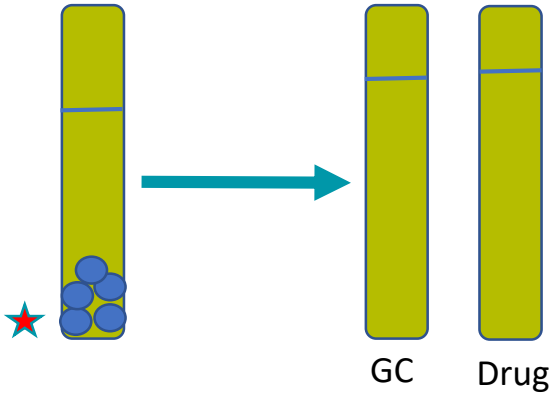
100 µL drug

# Using inoculum from positive MGIT: CL3

- Once a MGIT has flagged positive at or after 4 days, it must be used within 1-5 days
- If a MGIT is unloaded on day 0, the tube should be re-incubated for a minimum of one day (day 1)

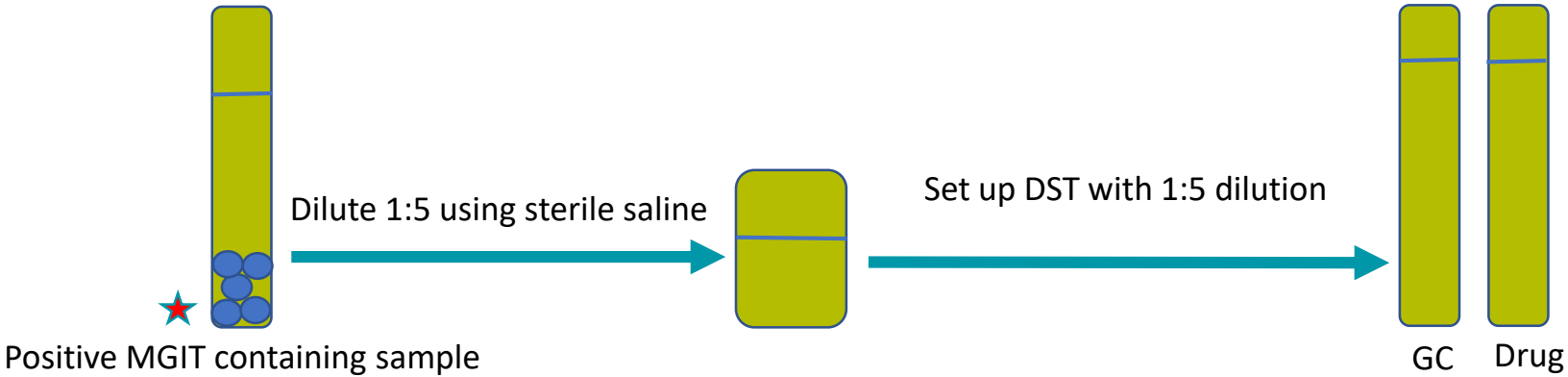
# Using inoculum from positive MGIT: CL3

## **Day 1** and **Day 2**




- Add glass beads
- Vortex 2-10 min
- Leave to settle for 30 min

## **Day 3**, **4 and 5**



# Growth Control Preparation: CL3

- SIRE/MOX/KAN drug susceptibility sets require the organism suspension to be diluted 1:100 for the growth control from which 500 µL is inoculated

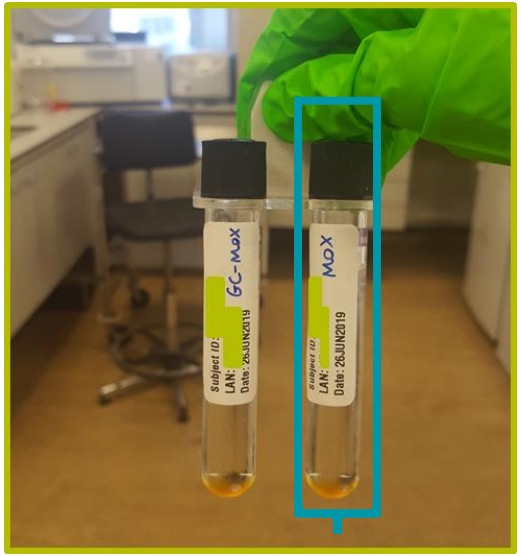
- PZA drug susceptibility sets require the organism suspension to be diluted **1:10** for the growth control from which 500 µL is inoculated


Organism Suspension diluted 1:100 for GC

500 µL inoculated

# Inoculation of tubes: CL3

- 500 µL of the organism suspension is inoculated into the drug tubes
- A blood agar plate is used to check for contamination (Incubated at 35-37°C and checked at 48 hr)




500 µL organism suspension inoculated

# Loading DST into BD BACTEC MGIT machine

- SIRE/MOX/KAN have a time in protocol of 4-13 days
- PZA allows for a longer timeframe of 4-21 days

# Drug Susceptibility Testing Principle Overview

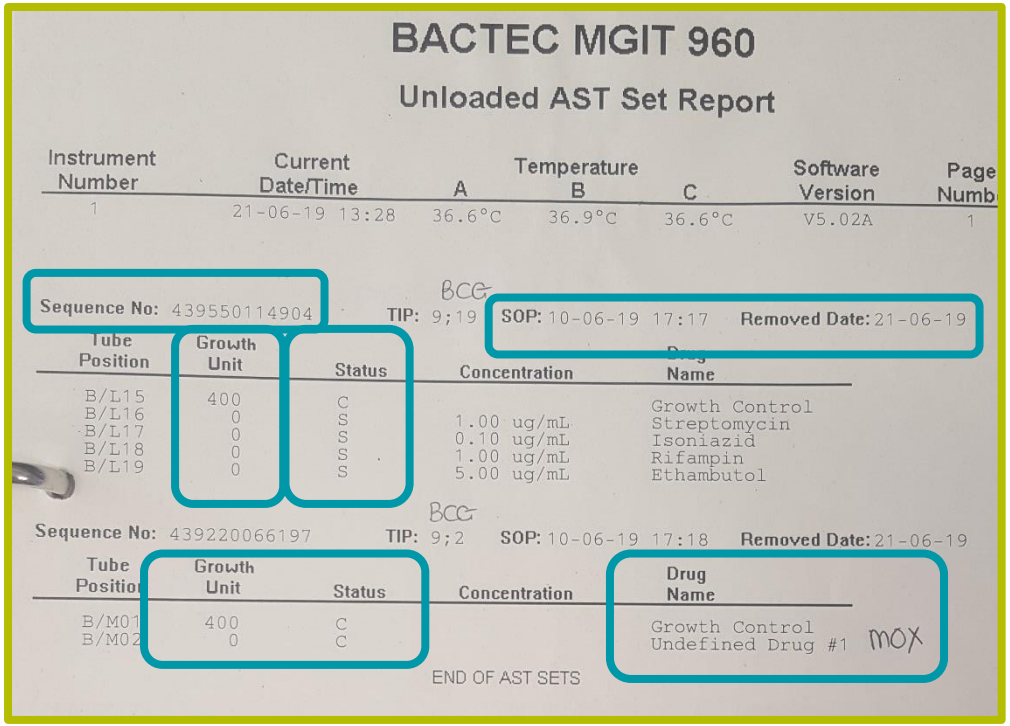
**Susceptible result:**

- Growth will be inhibited and fluorescence will be suppressed in the drug-containing tube
- The drug-free growth control will grow and show increasing fluorescence
- Sensitive result: Growth units are less than 100

**Resistant result:**

- Growth and its corresponding increase in fluorescence will be evident in both the drug-containing and the drug-free growth control tube
- Resistant result: Growth units are more than 100

# Interpreting DST results



BACTEC MGIT 960

Unloaded AST Set Report

| Instrument Number | Current Date/Time | Temperature A | Temperature B | Temperature C | Software Version | Page Numb |
|---|---|---|---|---|---|---|
| 1 | 21-06-19 13:28 | 36.6°C | 36.9°C | 36.6°C | V5.02A | 1 |

BCG

Sequence No: 439550114904 TIP: 9;19 SOP: 10-06-19 17:17 Removed Date: 21-06-19

| Tube Position | Growth Unit | Status | Concentration | Drug Name |
|---|---|---|---|---|
| B/L15 | 400 | C | | Growth Control |
| B/L16 | 0 | S | 1.00 ug/mL | Streptomycin |
| B/L17 | 0 | S | 0.10 ug/mL | Isoniazid |
| B/L18 | 0 | S | 1.00 ug/mL | Rifampin |
| B/L19 | 0 | S | 5.00 ug/mL | Ethambutol |

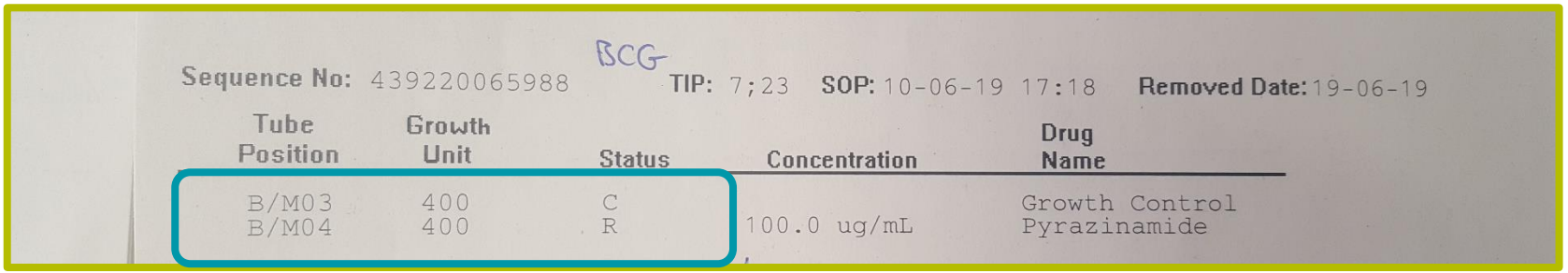
BCG

Sequence No: 439220066197 TIP: 9;2 SOP: 10-06-19 17:18 Removed Date: 21-06-19

| Tube Position | Growth Unit | Status | Concentration | Drug Name |
|---|---|---|---|---|
| B/M01 | 400 | C | | Growth Control |
| B/M02 | 0 | C | | Undefined Drug #1 mox |

END OF AST SETS

# Confirming Resistant Results



BCG

Sequence No: 439220065988 TIP: 7;23 SOP: 10-06-19 17:18 Removed Date: 19-06-19

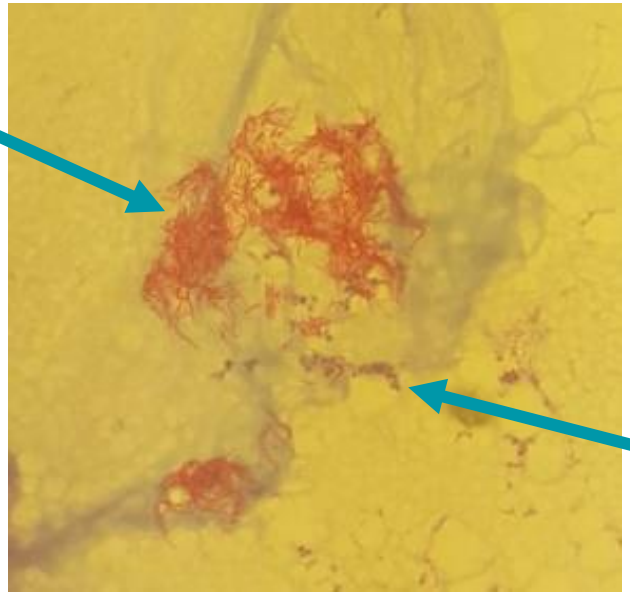
| Tube Position | Growth Unit | Status | Concentration | Drug Name |
|---|---|---|---|---|
| B/M03 | 400 | C | | Growth Control |
| B/M04 | 400 | R | 100.0 ug/mL | Pyrazinamide |

1. Blood agar culture (BAC) prepared - Check at 48 hr
2. Breadcrumb morphology check (no turbidity in MGIT)
3. Staining – ZN or Kinyoun (if needed)

# Confirming Resistant Results

| | |
|---|---|
| **Resistant Result Accepted** | BAC has no growth |
| | Colony morphology is typical |
| **Resistant Result NOT Accepted** | BAC shows growth |
| | MGIT does not show typical morphology |
| | Smear shows contamination |

# Confirming Resistant Results




Kinyoun stain showing contamination

# Error Messages

- If AST print out shows an X
- Run has failed – GC reached 400 GU outside of acceptable timeframe
- The result is invalid and no interpretation (S/R) will be shown

# Error Message X200

- System cannot detect sufficient growth in GC tube in specified protocol time:

1. Too little inoculum
2. Non-viable organisms
3. Slow growing drug-resistant strain

- On repeat sub-culture, use 3-5 days undiluted culture

# Error Message X400

- Detects indications of possible contaminated or over inoculated GC tube
- Perform BAC to check tube is pure
- On MGIT repeat sub-culture, use between 1-2 days

# Quality Control

- Perform DST testing on H37Rv (or equivalent fully sensitive isolate)
- Each batch of reagents (drug kits and tubes) must be quality tested
- If the QC fails: all results for batch must be reviewed, new reagents purchased, and testing of samples repeated

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