THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

Training on Tuberculosis Drug and Susceptibility Testing (MGIT DST – Liquid Method)

Module 10: Interpretation and reporting of MGIT 960 DST

Date:

By:

Venue:

Learning objectives

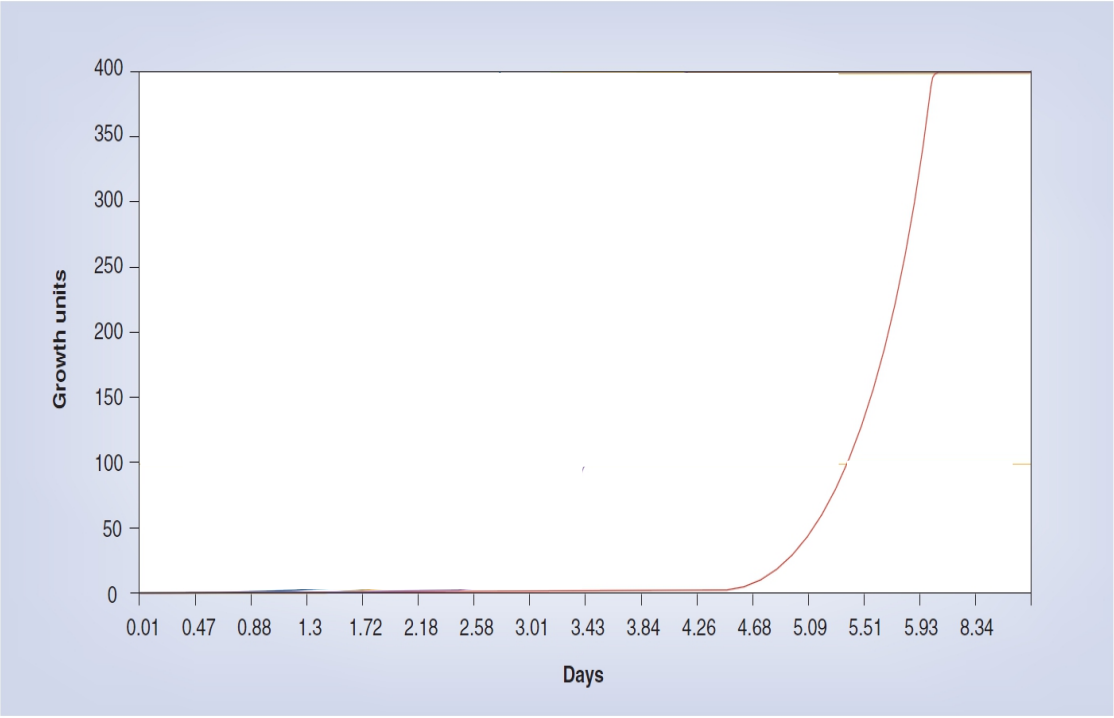
- Understand the automatic quantitative read and qualitative interpretation of MGIT DST results
- Understand how to validate MGIT DST results
- Understand how to record MGIT DST results in the laboratory register
- Understand how to report a *M. tuberculosis* complex MGIT DST on laboratory form

Module outline

- Reading MGIT 960 DST
  - Principle
  - Workflow steps
  - Unloaded AST set report
- Validation of DST results
  - Resistance
  - Unexpected result
- Laboratory register
- Laboratory report

Principle of reading MGIT 960 DST (1)

- The BACTEC MGIT 960 instrument continuously monitors the fluorescence of tubes in terms of Growth Units (GU, quantitative)



Growth curve of a *M. tuberculosis* strain

control

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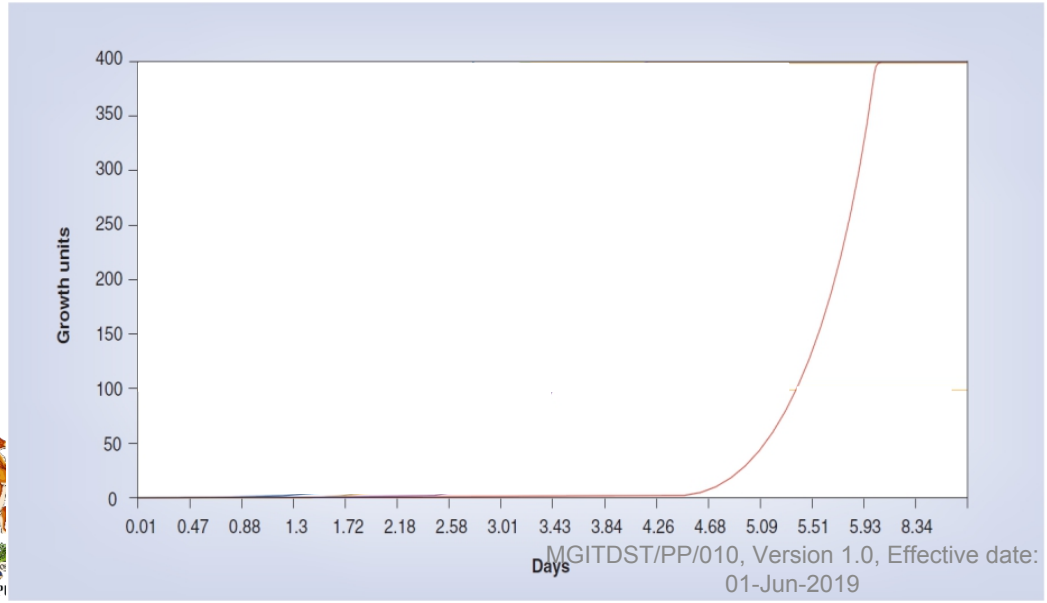
Principle of reading MGIT 960 DST (2)

- When the growth control reaches a GU of > 400 between 4–13 days for SIRE and 2nd Line drugs and 4–21 days for PZA: predefined algorithms compare the GU of drug containing tubes with the growth control tube



Growth curve of a *M. tuberculosis* strain

control

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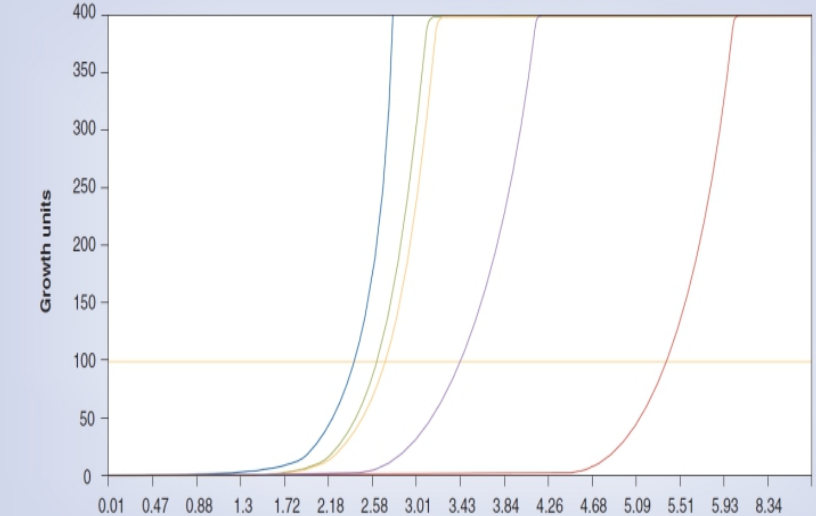
Principle of reading MGIT 960 DST (3)

- Growth Control GU reading is 400 for valid test
- Results are qualitatively interpreted from the GU of the drug-containing tube
  - GU < 100: interpreted as "Susceptible" (S)
  - GU > 100: interpreted as "Resistant" (R)

Example of MTBC strain

- susceptible to EMB
- resistant to INH-RIF-SM



Growth curve of a *M. tuberculosis* strain

- control
- isoniazid
- streptomycin
- rifampicin
- ethambutol

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Workflow steps of reading MGIT 960 DST (1)

- Once the test is complete, the instrument indicates that the results are ready
- Remove completed DST tubes
  - Open the desired drawer and press the “remove completed AST sets” soft key
  - The first completed DST set stations illuminate with FLASHING GREEN indicators
  - Remove the carrier and

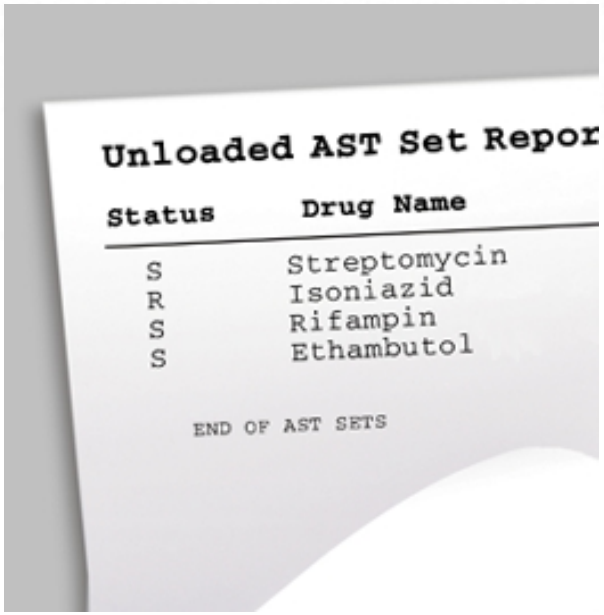
Workflow steps of reading MGIT 960 DST (2)

- Recheck that the GC is left-most of the tube set, and that the placement of drug-containing tubes is in expected sequence

- Print the result in the Unloaded DST set report
  - One report per page
- Repeat to remove additional DST sets

OR

- Print more than one DST report per page
  - Remove all completed DSTs



Reading an unloaded DST set report

BACTEC MGIT 960
Unloaded AST Set Report

Instrument Number	Current Date/Time	Temperature A	Temperature B	Temperature C	Software Version	Page Number
1	01/16/98 04:30	36.5 C	37.1 C	36.8 C	V3.XXY	1

Sequence Number: 439500000000 TIP: 7; 2 SOP: 01/10/98 08:22 Removed Date: 01/16/98

Access No: 1313 Isolate No: 1

Tube Position	GU	Status	Concentration	Drug Name
A/A01	400	C		Growth Control
A/A02	7	S	1.00 ug/mL	Streptomycin
A/A03	0	S	0.10 ug/mL	Isoniazid
A/A04	16	S	1.00 ug/mL	Rifampin
A/A05	0	S	5.00 ug/mL	Ethambutol

END OF AST SETS

Reading a unloaded AST set report

- GU values around 100 in the drug-containing tube
- The isolate can have a borderline resistance (typically a MIC near the test concentration)

- The instrument will interpret strictly according to the rule
  - GU = 99, interpretation is Susceptible
  - GU=100, interpretation is Resistant
  - Record GU for drugs on worksheet

- Review history of DST results for patients and examine borderline resistance

Status X: error/Indeterminate results (1)

- **INVALID SETS** (continued)
  - The instrument ALWAYS calls the ENTIRE DST SET invalid, NEVER one tube within the set.
  - The report shows sub-codes (for the E92 DST set error) printed beneath the respective tube within a set:
    - 0010 vial missing
    - 0200 not sufficient growth in GC within 13 or 21 days
    - 0400 over-inoculation or contamination
    - (See BACTEC MGIT 960 Systems User's Manual for a complete list of error codes)

Status X: error/Indeterminate results (2)

- Invalid sets: What to do?
- 1. Print an Inventory Control report for the drawer(s) with errors before scanning out tubes
  - Inventory control report prints GU for GC drug tubes
  - Determine status of tubes
    - Is Growth Control GU value
    - Drug tubes GU values
  - Cannot print inventory control if tubes are removed (unless Epi Centre is installed)

Status X: error/Indeterminate results (3)

- Repeat all invalid DST results
  - Inoculate new MGIT seed for DST

- Evaluate GU for GC and drugs on failed DST set
  - If the GC was not zero, look at age of MGIT tube when DST was set. A 1:5 dilution on day 3 may give a lower bacterial concentration. Consider resetting the DST set on Day 2 (undiluted) or wait for Day 5 (diluted) to have more bacterial load
  - GC with GU = 0, may not be inoculated

Validation of DST results (1)

- In case of resistance
- make sure that the culture it is not contaminated by NTM or other bacteria (check the medium visually, look for turbidity, ZN smear, purity on blood agar and 7H10 plates)
- In case of mono-resistance against RIF, PZA or EMB
- repeat the test to verify resistance before reporting
- Mono-resistance to PZA
- is uncommon but *M. bovis*, including *M. bovis* BCG, is naturally resistant to PZA
- In case of a new patient isolate found resistant
- reconfirm the resistance by the same or different method
- In case of unexpected results
- repeat the test

Validation of DST results (2)

- In case of resistance to the critical concentration of INH, SM or EMB: option to test higher concentration

Drug	Critical µg/mL	High µg/mL
S	1.0	4.0
I	0.1	0.4
R	1.0	-
E	5.0	7.5

- to consider low-level resistance if resistant at the low level and susceptible at the high concentration (CLSI)
- isolates with low-level resistance repeatedly tested with the same method may give inconsistent results, or give discordant results when compared with another method
- clinical relevance of higher concentration drug test is not defined

Recording DST results (1)

- **DST and identification register**
- Primary culture serial number
- Date culture positive
- DST serial number
- Date DST inoculation

- **DST logbook**
- Control /Drugs
- Identification tests
- **Final results**
- Identification
- Resistance profile
- Date of report

Recording DST results (2)

- Note results immediately on DST worksheet and Laboratory register.
- Always check agreement between lab serial number on tube and number in laboratory register.
- Check for clerical errors!

Reporting DST results

DST Reports have to specifically indicate:

•the qualitative result: “Susceptible” or “Resistant”

MGIT First Line DST Result Worksheet

Tech. Name:_____ Date:_____

Fill in Table below completely

No.	NTRL Lab.	Streptomycin	Isoniazid	Rifampicin	Ethambutol	Pyrazinamide	Comments
1							
2							
3							
4							

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INH higher concertation tested

- OPTIONAL – Laboratory dependent. Report only if a higher Concentration of INH is done

- If INH resistant at low concentration, send a preliminary report

- *"Test indicates the presence of low-level INH resistance. Testing at the higher level of INH will be performed. A specialist in the treatment of tuberculosis should be consulted concerning the appropriate therapeutic regimen and dosage."*

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Turn-around time for DSTs

- DST reports available
  - within 15–30 days from specimen reception
  - within 7–14 days after isolation of MTBC
- Report validated DST results as soon as they are available
  - by telephone/fax/computer/messenger with a follow-up hard copy report
  - to the requesting physician, infection control staff, and the local tuberculosis control program
- Quality Assurance Indicator
  - Track number of days for each final DST report

Reading MGIT 960 DST: summary

- Instrument monitors DST set [drugs and Growth Control (GC) tubes]
- Interprets susceptibility automatically (4–13 days)
- When Growth Control GU reaches 400, the instrument evaluates drug tube
  - GU Drug ≤100 = Susceptible
  - GU Drug >100 = Resistant
  - GU around 100 = usually borderline (interpreted S or R)
- Results automatically interpreted: S or R
- If too heavy or too light inoculum: Error 'X' (indeterminate)
- The Instrument Inventory Report prints GU values for all ongoing DST sets

Assessment

1. What is the principle of reading MGIT DST?
2. What are cut off growth control units for reading growth control and drug tubes?

Exercise

- List the possible causes of X400 and X200 errors and possible ways to mitigate them.

References

- BACTEC® MGIT 960™ System User's Manual. Becton Dickinson Company. 2004/06 Document number MA-0117. Revision E
- Global Laboratory Initiative (GLI) http://www.gliquality.org/
- www.who.int/tb

Acknowledgments