

# **Role of liquid media in diagnosis and drug susceptibility testing of Mycobacteria**

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# FACTS..

- **Estimated 424,000 MDRTB cases each year**
- **Approx 25,000 (6%) are expected to have XDRTB**
- **Laboratory play a critical role in identifying MDR & XDR**
- **Less than 5% of MDRTB cases are currently detected**
- **Laboratory must develop capacity to diagnosis by use of rapid method**

# Rapid Diagnosis

- **Rapid diagnosis enables rapid treatment**
- **Rapid antimicrobial treatment shortens disease course and minimizes risk of disseminated (miliary) TB**
- **Rapid treatment minimizes spread of infection – public health impact**

# Mycobacterial Species

- **Runyon (1954) Classification of Mycobacteria**
- ***M.tuberculosis* Complex and NTM**
- **Non-pathogen and Potential pathogen**
- **More than 100 species have been identified.**
- **Almost any species can cause infection in immunocompromised patients.**

# AFB Microscopy

- **The Best for Diagnostic Test with good EQA**
- **Sensitivity only 30-60%**
- **Can not differentiate species**
- **Don't know dead or live bacilli**
- **Limitation of 10,000 cell/ml**
- **If diagnosis by smear, 50% TB patients are missed**

# Culture

- **Culture is the Only Definitive diagnosis**
- **Culture is necessary for ID and DST**
- **Culture is going to remain as a “Gold Standard” for a long time**
- **Culture on solid media are take time (6 – 8 weeks) and insensitive**
- **Sophisticated facilities and expertise**

# Liquid Media

- **Liquid Media are far superior to Solid Media**
- **Rapid turn around time**
- **More mycobacterial species grow**
- **Stressed mycobacteria grow better**
- **Rapid and more Reliable Susceptibility test**

# Efficiency of Culture

- **Process of fresh specimen**
- **Time from collection to process for culture**
- **Prevent growth of contaminants by keeping at cold temperature in transit and storage**
- **Decontamination method: Mild enough, not to harm MTB but to remove all contaminants**
- **Concentration of bacilli**
- **Growth medium and environment**



# Changing Trends in Laboratory Testing

- Days of Home-made media, reagents and procedures are gone.
- Commercial test kits, procedures and reagents are more widely used.
- Automation is taking place of manual testing.
- There is a demand of Rapid Testing, Rapid Reporting for “Better Patient Care”

# **Impact of New Technologies**

- **Standardized procedures**
- **Tested Quality Controlled Media and Reagents**
- **Simple to perform**
- **Labor saving**
- **Shorter Turn Around Time**
- **Good Quality Control**
- **High sensitivity & specificity**

# Recommendation for Laboratory Service (1)

- Prompt delivery of specimens to the laboratory.
- Use of rapid, State-of-the-Art methods (e.g., fluorescence microscopy, liquid media, and rapid identification methods).
- Reporting of smear results to health-care providers within 1 day.
- Reporting of culture identification of *M. tuberculosis* complex within 21 days from specimens collection.

# **Recommendation for Laboratory Service (2)**

- Reporting of drug-susceptibility test results within 30 days.**
- Reporting of all positive test results to the specimen submitter within 1 working day from the date of report.**
- Initial isolates from all patients should be tested by both solid & liquid medium**

# CDC Recommendations

- **Use most rapid methods available for diagnostic mycobacterial testing, including susceptibility testing**
- **Initial isolates from all patients should be tested by both solid & liquid medium**
- **Reports of drug susceptibility tests should be available within 15-30 days from specimen collection**

**Bactec system, Non-  
Radiometric Liquid Media, has  
the potential to provide this  
suggested turnaround time**

# The New Culture Systems 1990....

## Non-Radiometric Liquid media

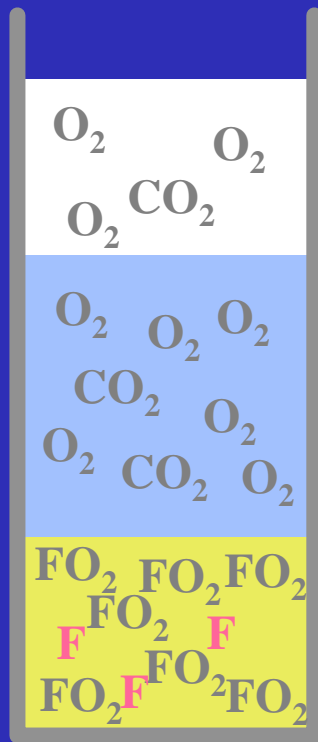
- MGIT Manual (BD) - *Detection & DST*
- BACTEC MGIT 960 (BD) - *Detection & DST*
- MB/BacT, BacT Alert 3D (Biomერიux)  
- *Detection only*

*Which one to use ??*

Courtesy by: S. Side

# Indicator System

## Negative Culture



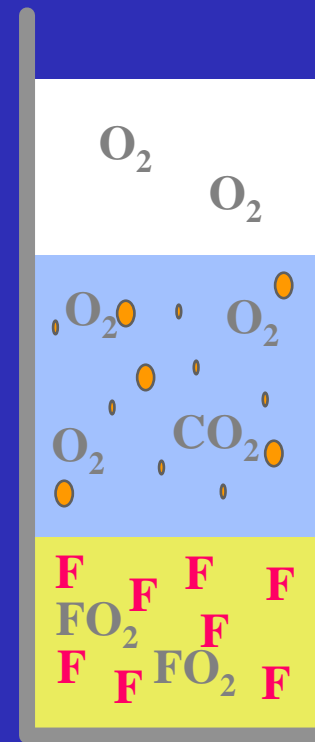
Headspace

Broth

Sensor

Little or No Fluorescence

## Positive Culture

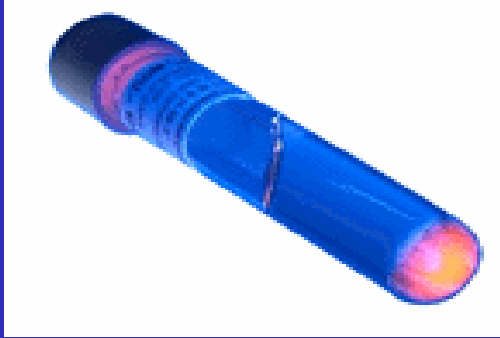


Strong Fluorescence



# BACTEC MGIT 960 SYSTEM

- **Continuously monitored**
- **Easy to use system with fewer handling requirement**
- **Use screw cap and needle inoculation is not necessary.**
- **Occupied less space in laboratory.**



# **BACTEC MGIT 960**

- **Same/Better performance as BACTEC 460**
- **Plastic screw-cap tubes. No glass, No needles**
- **High volume. One instrument incubates 960 tubes, up to 8000 specimens / year**
- **Automated, Continuous monitoring  
Identifies positive results**

# **Diagnosis of TB in HIV infected person**

- Well known that difficulties in Dx**
- Use liquid media in case of smear negative, not all cases**
- Show abnormal shadow on CXR**

# Rapid detection of Mycobacteria Using MGIT – Thailand

Site	N	Median (days)
Bangkok	96	10
Chiang Rai	72	7
Phuket	75	9
Ubon	87	8

# Recovery Rate of LJ vs. MGIT

## Bangkok, 2004 - 2006

<b>Characteristic</b>	<b>All N (%)</b>	<b>M+ N (%)</b>	<b>M- N (%)</b>
<b>No. of specimens</b>	<b>3051</b>	<b>1394</b>	<b>1657</b>
<b>Growth on at least LJ</b>	<b>1130 (37)</b>	<b>539 (39)</b>	<b>591 (35)</b>
<b>Growth on at least MGIT</b>	<b>1541 (50)</b>	<b>732 (53)</b>	<b>809 (50)</b>
<b>Growth on both LJ and MGIT</b>	<b>1062 (35)</b>	<b>504 (36)</b>	<b>558 (34)</b>

**Liquid Culture, MGIT, Performs Well  
in Routine Practice in a High-Burden  
TB Country**

**Increased sensitivity & speed**

**BUT**

**Lack of visual evaluation of MTB & NTM**

# Identification

- Subculture .....3 – 4 weeks
- Morphological examination
- Biochemical test
  - Niacin
  - Nitrate reduction
  - Catalase 68°C , 20 mins
  - Others test ect. (Take a weeks)

**BUT**

AccuProbe can solved...(Take 2 days)

**New Novel Technology..**

**Capilia TB Test** can identification  
*M. tuberculosis* with in 15 min!!



# Capilia TB: A Rapid Identification Test

*(ICA test kit for MPB64)*

- Immunochromatographic detection of species-specific antigens
- MPB64, identified in 1984 as *Mycobacterium tuberculosis* complex-specific secretory protein, has been used as the target antigen in a simple lateral flow speciation test using anti-MPB64 monoclonal
- No sample processing or instrumentation is required

Positive cultures of *Mycobacterium* spp.



Capilia TB test



Source: Dhanida Rienthong, NRL/ SRL of Tuberculosis, Thailand

# What is the normal acceptable contamination rate?

**Solid media**      **5% ± 2 %**

**Liquid media**      **slightly higher  
contamination rate is  
expected (7 - 8%)**

**In Vietnam NRL**      **3.96%**

**In Thailand NRL**      **4.74%**

# DST by “In house” Culture System

- **Must be validated locally**
- **Typically base on solid media**
- **Drug / Drug solution source of error**
- **Inspissation of egg media critical**
- **Short shelf-life of drug media**
- **Cheaper consumable**
- **Labour expensive**

# **DST by Commercial Culture System**

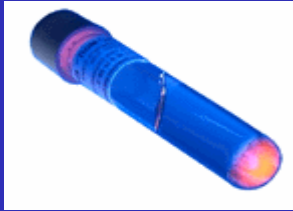
- **Validated and generally reliable**
- **Typically based on liquid media**
- **No need to prepare drug solution**
- **Culture tube cost?**
- **May require expensive equipment**
- **Technical support from supplier**

# **BACTEC MGIT 960 AST SIRE**

- 1. Fully automated DST machine.**
- 2. Used as rapid qualitative procedure for DST from culture.**
- 3. Automatically interpret the results.**
- 4. Reported as Susceptible or Resistant in a week.**

# Drug Susceptibility Testing

## BACTEC MGIT 960



- **Drugs: SIRE & PZA**
  - All drugs FDA Cleared
  - Meets recommendations of NCCLS  
(low and high concentrations for SIE)
  - Secondline DST. Multicenter Study in Europe
- ***The only automated system that offers susceptibility testing of 5 drugs***

NCCLS = National Committee for Clinical Laboratory Standard

# **BACTEC™ MGIT™ 960 vs BACTEC™ 460TB DST**

## **Time-to-Result from 3 published studies**

	<b>BACTEC 960</b>	<b>BACTEC 460TB</b>
<b>F. Ardito, et al</b>	<b>7.9 days</b>	<b>7.3 days</b>
<b>P.Bemer, et al</b>	<b>6.5 days</b>	<b>7.0 days</b>
<b>E.Tortoli, et al</b>	<b>9.4 days</b>	<b>6.9 days</b>

# Why Expand Liquid Culture Capacity?

- **Smear microscopy is insufficient.**
- **To demonstrate feasibility and effectiveness of mycobacterial culture.**
- **To strengthen diagnosis of TB and MDRTB in HIV- epidemic area**
- **To use for rapid decision making of treatment**
- **Benefit for control of MDR/XDR?**



# Conclusion

- **MGIT liquid media appear to be rapid, reliable and easy to use for detecting viable mycobacteria directly from clinical specimens.**
- **Automate Bactec system perform for testing DST are accurate and rapid for DST of *M.tuberculosis*.**
- **Use liquid media both isolation and DST will be benefit for control of MDR / XDRTB.**

**Thank you  
for your Attention**



**Welcome to Thailand**