

**NIMR - MMRC**



NIMR-MMRC  
Mbeya Medical Research Center

**LMU**

**KLINIKUM**

DER UNIVERSITÄT MÜNCHEN

ABTEILUNG FÜR  
INFEKTIONS- UND TROPENMEDIZIN  
MEDIZINISCHE POLIKLINIK INNENSTADT  
LEITER: PROF. DR. MED. M. HOELSCHER



Event: The 7th EAHSC;

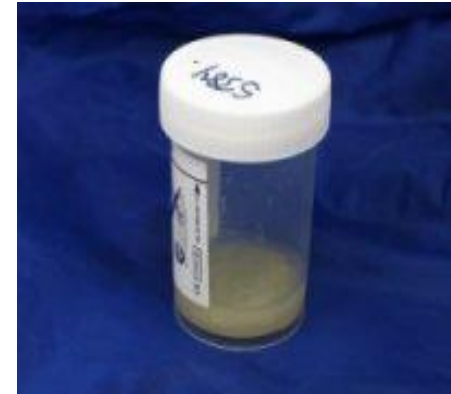
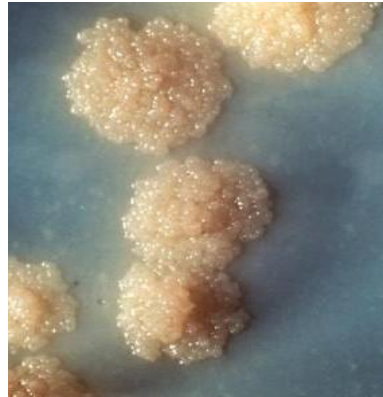
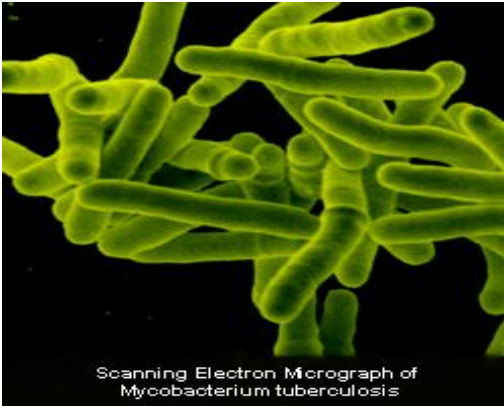
Utilization of the Molecular Bacterial Load Assay for the Detection of 16s M.tb rRNA in sputum sample and Monitoring treatment response to the TB patients by PCR (RT-qPCR)

**Daniel Adon Mapamba Msc.<sup>1</sup>**, Bariki Mtafya,<sup>1\*</sup> John Joseph<sup>1</sup>, Nyanda Elias Ntinginya,<sup>1\*</sup> Norbert Heinrich<sup>†\*</sup>

<sup>1</sup> National Institute of Medical Research, Mbeya Medical Research Center, Tanzania;

<sup>†</sup> Division of Infectious Diseases and Tropical Medicine Munich, \* Co-authors

# Introduction on *Mycobacteria tuberculosis (M.tb)*

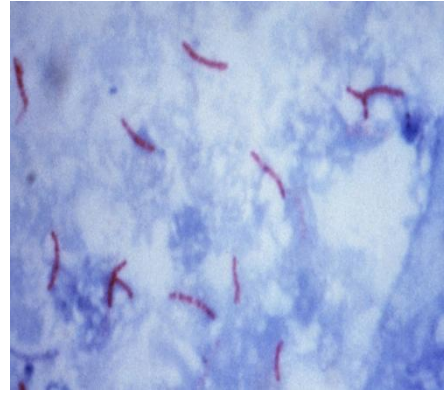
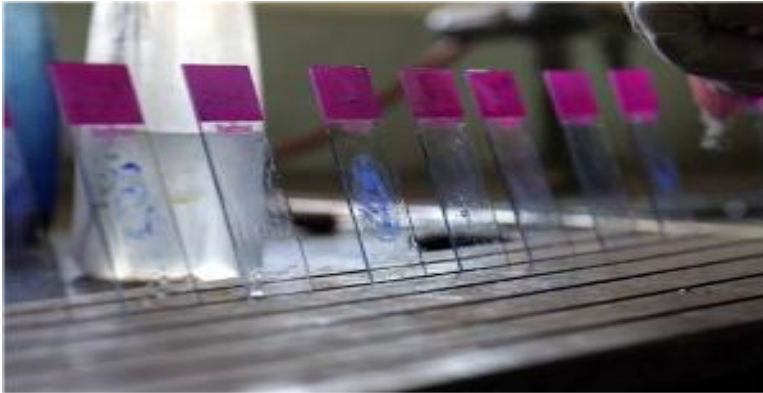


*Mycobacterium tuberculosis (M.tb)* is an obligate pathogenic bacteria, the causative agent of tuberculosis (TB) disease.

The disease spread from person to person by aerosol route and causes about 2 million death annually.

- ➔ Advanced diagnosis and effective monitored treatment is needed to alleviate the disease
- ➔ Sputum is the most commonly used biological sample for disease diagnosis.

# Current methods of M.tb detection: smear microscopy



## Limitations of smear Microscopy

- Many patients' sputa are smear negative, while they still have M.tb (sensitivity varies between 35-80% in routine clinical practice).
- As low as 20% among HIV-infected patients.
- There is a limit of detection;  $10^4$  bacteria required to detect by smear.
- Detect both live and dead cells

# MGIT – Mycobacteria growth indicator tube system



## Advantages

- ✓ Fully automated system
- ✓ Gold standard method
- ✓ Can be used to monitor treatment response

## Disadvantages

- ✗ Expensive
- ✗ Prone to contamination
- ✗ Decontamination kills some bacilli



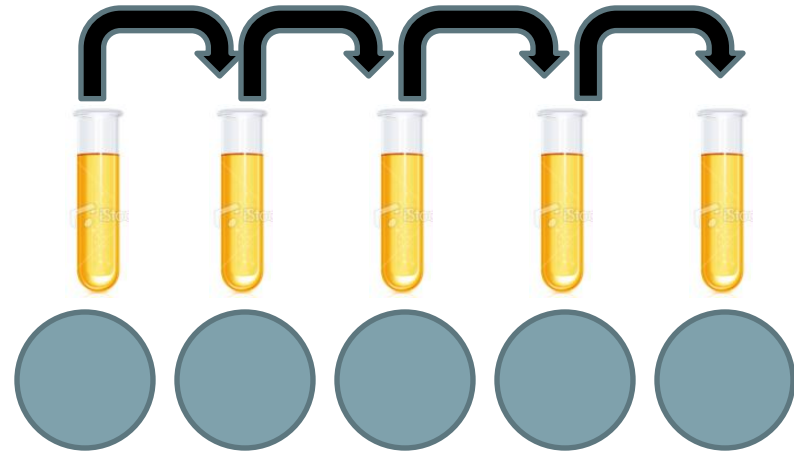
- ✓ 1<sup>st</sup> automated rapid, sensitive molecular platform for diagnosis and simultaneously detection of MDR cases

- ✗ Not useful for monitoring treatment response


# Serial colony count by agar plate, CFU

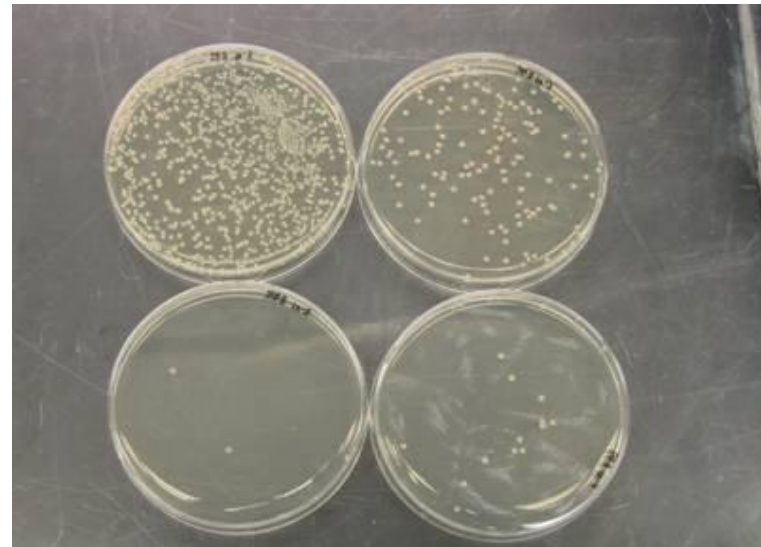
## Advantages

- ✓ Gold standard method
- ✓ Useful to monitor treatment response
- ✓ Quantitatively detect viable bacilli



## Disadvantage

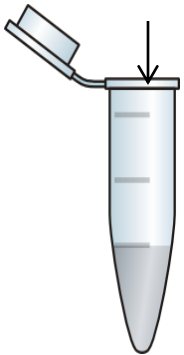
- ✗ Laborious 
- ✗ Time consuming
- ✗ Low-throughput
- ✗ Prone to contamination



# Molecular Bacterial Load Assay

- The assay uses RNA as a starting material
- RNA are extracted from sputum preserved with GTC +  $\beta$ -Me by qiagen protocol

EC+ Sample



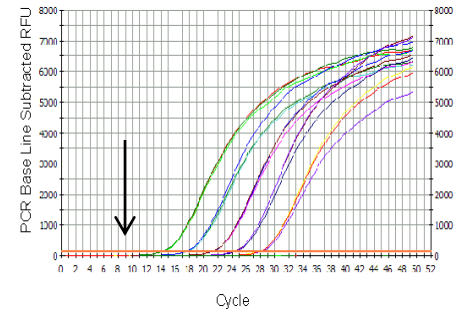
RNA Extraction



one step RT-qPCR



Data analysis



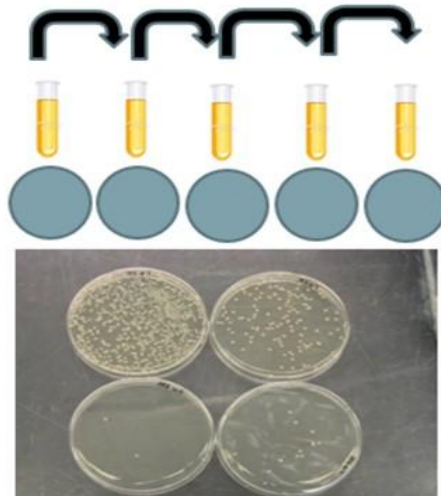
- Quantitect RT-qPCR : MBL is run in a duplex reaction, using 2 different probe/primer sets, one set for Internal control and the other for 16S rRNA gene

# Specific Objectives

- To validate the new diagnostic technique that can be able to timely detect M.tb and monitor treatment response to the TB patients who are under medical care
- Comparing time to results between MBL assay and conventional diagnosis methods

# Practical set up

- Total of 255 sputum samples, obtained from PanACEA TB studies at NIMR-Mbeya were used for MBL assay validation in parallel with conventional methods.
- 213 samples were used to set MBL and MGIT culture only 42 used for Agar plate
- 5 ml frozen samples were thawed and spiked with 100 ul of Internal control prior to RNA extraction procedures





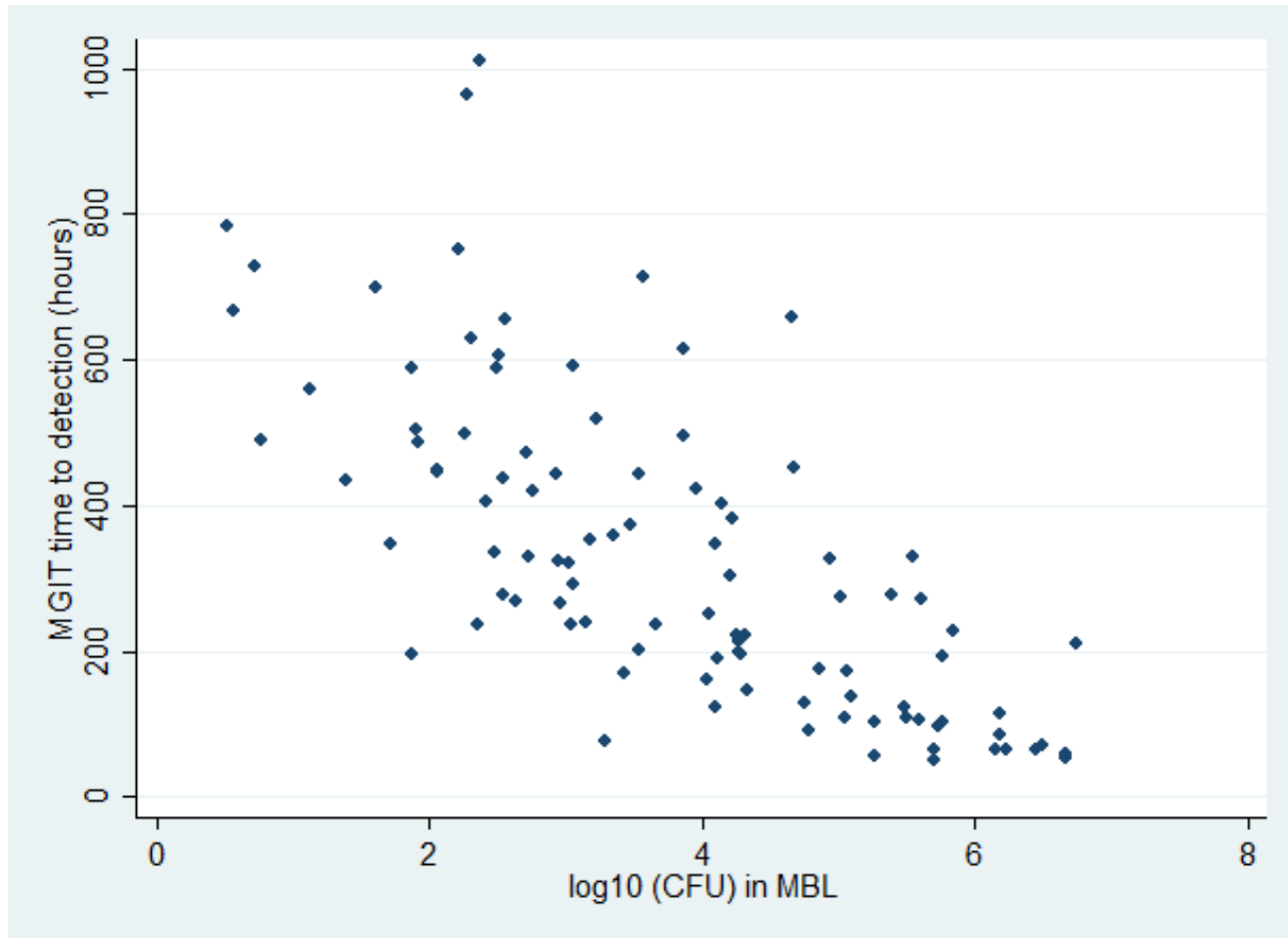
# Relationship between MGIT and MBL assay on M.tb detection

	<b>MBL negative</b>	<b>MBL positive</b>	<b>Total</b>
<b>MGIT negative</b>	26 (41.94%)	23 (15.23 %)	49 (23.00%)
<b>MGIT positive for TB</b>	12 (19.35 %)	89 (58.94 %)	101 (47.42 %)
<b>MGIT positive for TB and for contamination</b>	0 (0.00%)	4 (2.65%)	4 (1.88%)
<b>MGIT contaminated</b>	24 (38.71%)	35 (23.18%)	59 (27.70%)
<b>Total</b>	62 (100.00%)	151 (100.00%)	213 (100.00%)

# Relationship between solid culture and MBL assay on M.tb detection

Solid culture (agar plate)	MBL assay result		
	Negative	Positive	Total
Negative	3 (100%)	12 (30.8%)	15 (35.7%)
Positive	0 (0.00%)	27 (69.2%)	27 (64.3%)
Total	3 (100%)	39 (100%)	42 (100%)

# Quantitative correlation between MGIT a MBL



**Fig 1.** Spearman correlation at rho: -0.76 with strong negative correlation between MGIT and MBL assay of 213 samples

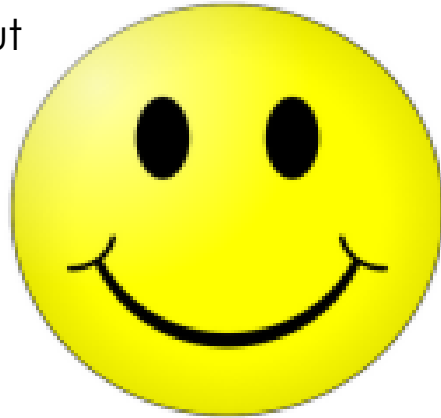


# Summary

✓ Medium- high throughput

✓ Avoid contamination

✓ Easy to perform



✓ Molecular based assay

✓ Rapid

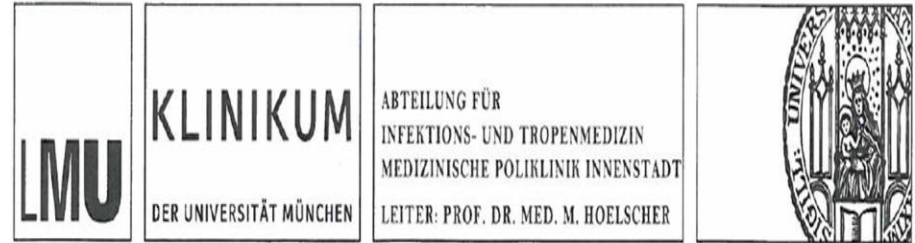
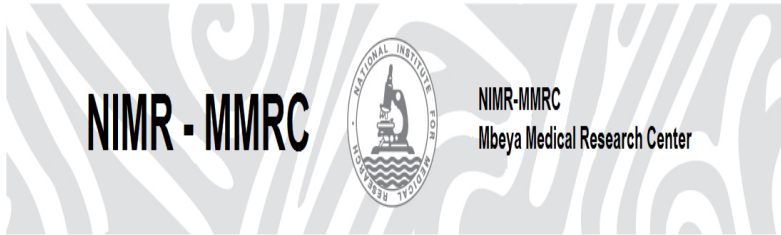
✓ Standard method under Predict TB

- ❖ MBL assay can be used to monitor treatment response to patients who are under medical treatment following its sensitivity and ability to identify viable M.tb
- ❖ Ability to detect viable-non culturable M.tb (M.tb in dormancy state)
- ❖ Conclusively; the MBL assay is a novel Molecular test useful for viability testing of M.tb following on technical advantages over culture methods.

# Outlook

- The assay lacks drug susceptibility testing of M.tb
- Require many consumables (tips and tubes)
- In future plan is to use the assay to detect M.tb from urine samples of co infected individuals,
- More thoughts should be focused on
  - ❑ Invention technologies i.e point of care testing and speciation methods

# Acknowledgements



University of  
St Andrews