

# Contemporary Microbiological Diagnostic Tests for Rapid Identification and Detection of Resistance of *Mycobacterium tuberculosis*

Oliver Taleski<sup>1</sup>, Vaso Taleski<sup>1</sup>

<sup>1</sup>Faculty of Medical Sciences, Univ. „Goce Delchev“ Shtip,  
Republic of Macedonia

Presenter's phone 389 78 604 932,

e-mail: [oliver.111672@student.ugd.edu.mk](mailto:oliver.111672@student.ugd.edu.mk)



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FUTURE PERSPECTIVES IN MEDICAL AND  
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Меѓународен симпозиум на Факултетот за  
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Повеќе

A blue-toned graphic featuring a DNA double helix, laboratory glassware, and a person in a white lab coat. The background includes faint text like 'COMPLETE' and 'DNA'.



# ***INTRODUCTION (1)***

- **Tuberculosis (TB) occurs in every part of the world as a top infectious disease killer.**
- **In 2014, 9.6 million cases (an estimated 1 million children) and 1.5 million deaths (140 000 children) from TB were registered. Over 95% of TB deaths occur in developing countries.**
- **TB incidence has fallen since 2000 and is now 18% lower, by an average of 1.5% per year.**



## ***INTRODUCTION (2)***

- **TB is a treatable and curable disease with a standard 6 month protocol of 4 antimicrobial drugs. In cases of multi-drug resistant TB (MDR-TB ) responds are very weak including the most effective second-line anti-TB drugs. About 480 000 people developed MDR-TB in the world in 2014.**
- **Standard anti-TB drugs have been used for decades, and resistance to a single anti-TB drug have been documented in every country worldwide.**
- **Multidrug-resistant tuberculosis (MDR-TB) is a form of TB caused by bacteria that do not respond to, at least, isoniazid and rifampicin, the 2 most powerful, first-line (or standard) anti-TB drugs.**



## **OBJECTIVE**

- **To give overview on contemporary microbiological diagnostic tests for rapid identification and detection of resistance of *Mycobacterium tuberculosis***
- **To present possibilities of microbiological diagnostic tests for identification and detection of resistance of *Mycobacterium tuberculosis* in National laboratory in Republic of Macedonia**
- **To present data of results of some methods for rapid identification and detection of resistance of *Mycobacterium tuberculosis***

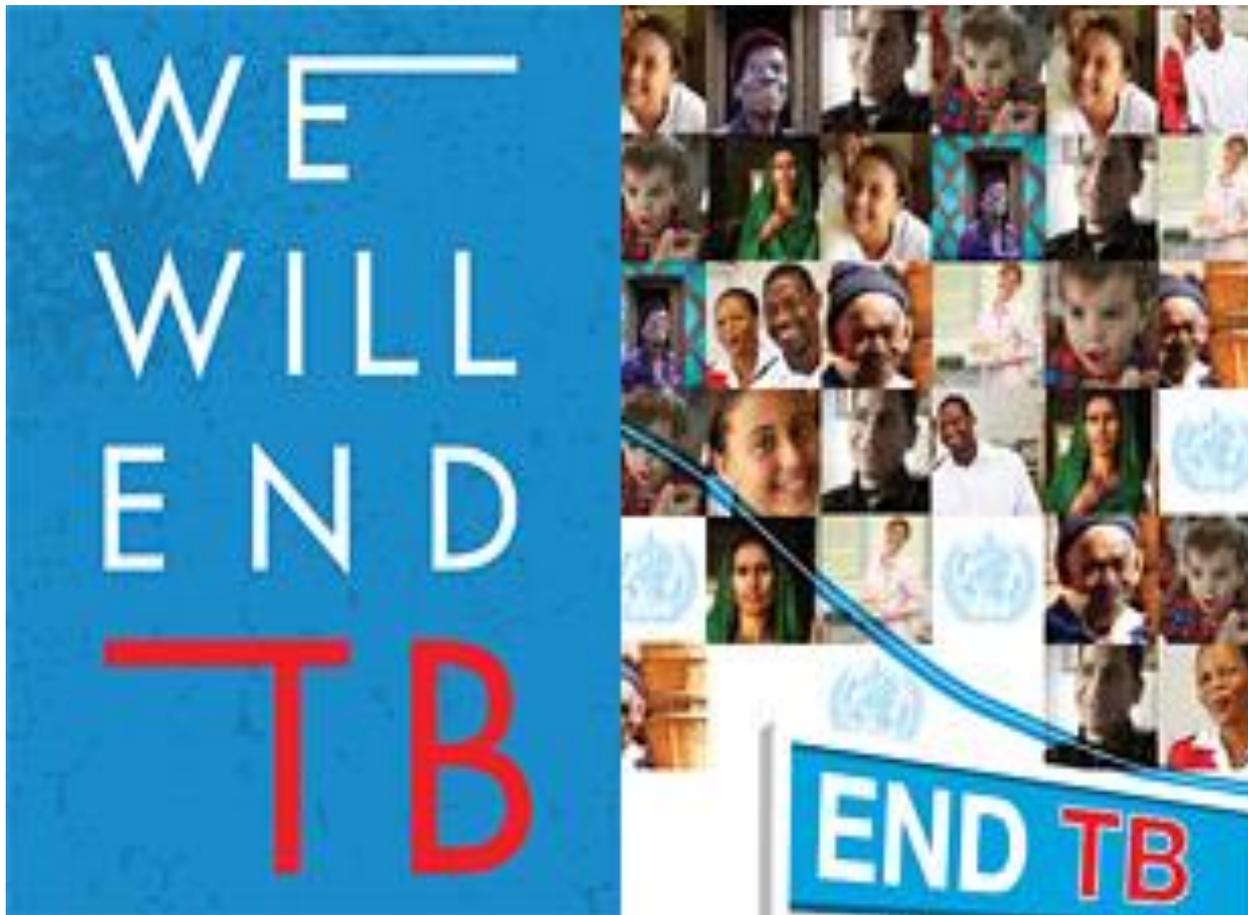
# ***MATERIAL and METHODS (1)***



- **Material and methods in use in National reference laboratory of Republic of Macedonia (microbiological laboratory at the Institute for lung diseases).**
- **Standard methods in use:**
  - **Culture on Löwenstein-Jensen medium**
  - ***Ziehl - Neelsen* staining/ or Cold staining- *Kinyon* method**
  - **Antituberculous drugs susceptibility testing on modified Löwenstein-Jensen medium for:**
    - ***Isoniazid (H) 0,2 µg/l***
    - ***Rifampicin ® 40,0 µg/l***
    - ***Ethambutol (E) 2,0 µg/l***
    - ***Streptomycin (S) 4,0 µg/l***



**Preparing staining and culture**



**WHO Goal target is ending the TB epidemic by 2030**



## ***MATERIAL and METHODS (2)***

- **Contemporary methods (1):**

- **Bactec MGIT 960**

Is an automated method enable identification of *Mycobacterium tuberculosis* for 4-12 -20 days, and susceptibility testing on : streptomycin, isoniazid, rifampicin, ethambutol and pyrazinamide, and tests for antituberculosis drug of second line.



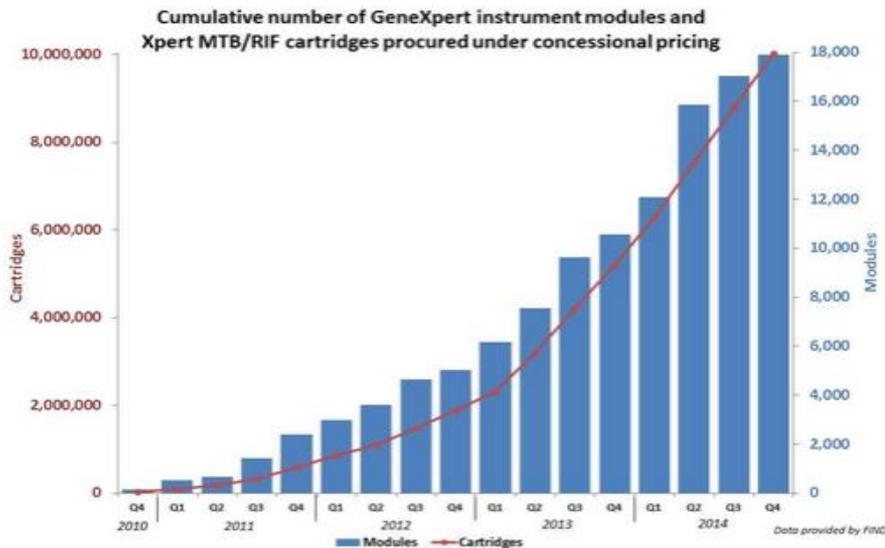
**Bactec MGIT 960**

# MATERIAL and METHODS (2)



- Contemporary methods (2):  
WHO monitoring Xpert MTB/RIF, managed by the WHO Global TB Program, is an automated, cartridge-based nucleic amplification assay for the simultaneous detection of TB and rifampicin resistance directly from sputum in under two hours. The technology is based on the GeneXpert platform

## Global summary statistics of procurement



As of 31 December 2014, a total of 3,763 GeneXpert instruments (comprising 17,883 modules) and 10,013,600 Xpert MTB/RIF cartridges had been procured in the public sector in 116 of the 145 countries eligible for concessional pricing.



## • **RESULTS**

Year	2010	2011	2012	2013	2014
Positive	502	350	428	376	375
Negative	5339	5594	5519	6060	6061

### **Culture results on Lövenstein-Jensen medium**

Year	2010	2011	2012	2013	2014
Positive	52	62	86	77	?
Negative	335	433	569	835	?

### **Bactec MGIT 960 results**

Year	2009	2010	2011	2012	2013	2014
<b>TB strains</b>	<b>191(100%)</b>	<b>181(100%)</b>	<b>155(100%)</b>	<b>181(100%)</b>	<b>198(100%)</b>	<b>154(100)</b>
<b>Sensitive</b>	<b>175(91,6%)</b>	<b>170(93,9%)</b>	<b>143(92,2%)</b>	<b>163(90%)</b>	<b>191(96,4%)</b>	<b>142(92,%)</b>
<b>Resistant</b>	<b>16 (8,3%)</b>	<b>11 (6%)</b>	<b>12 (7,7%)</b>	<b>18 (10%)</b>	<b>7 (3,5%)</b>	<b>12 (7,5%)</b>
<b>Mono-resistant</b>	<b>14 (7,3%)</b>	<b>3 (1,6%)</b>	<b>9 (5,8%)</b>	<b>11 (6%)</b>	<b>6 (3%)</b>	<b>10 (5,8%)</b>
<b>Rifampicin resistant</b>	<b>3 (1,5%)</b>	<b>/</b>	<b>5 (3,2%)</b>	<b>1 (0,5%)</b>	<b>1 (0,5%)</b>	<b>3 (1,7%)</b>
<b>MDR</b>	<b>1 (0,5%)</b>	<b>7 (3,8%)</b>	<b>1 (0,6%)</b>	<b>4(2,2%)</b>	<b>1 (0,5%)</b>	<b>2 (1,2%)</b>

## **TB resistance over period 2009-2014**

# CONCLUSIONS



- In National reference laboratory for Identification and Detection of Resistance of *Mycobacterium tuberculosis*, beside standard methods, contemporary rapid methods recommended by WHO experts and Global TB Program, such are: Bactec MGIT and GeneXpert are priority.
- Rapid methods enable final diagnosis of TB, including isolation, identification and susceptibility testing in significantly shorter time.
- Number of new TB infections in Republic of Macedonia is decreasing continuously in past 10 years.
- MDR strains in Republic of Macedonia are at low level.