Treating Patient, Not Disease: People-Centered Approach

7th TB Symposium – Ministry of Health of the Kyrgyz Republic and Médecins Sans Frontières

1-2 March, 2018, BISHKEK, KYRGYZSTAN

Treatment of MDR/XDR TB patients based on the results of the molecular-genetic diagnostics

I.A. Vasilyeva, A.G. Samoylova, A.E. Panova
Tuberculosis and Pulmonology Research Institute, Russian Federation
Tuberculosis incidence and mortality in Russia (per 100 thousand population) 1970 – 2016

Incidence

Mortality
Proportion of new MDR TB cases in Russia, 2006-2016

- 2006: 11.4%
- 2007: 12.9%
- 2008: 14.6%
- 2009: 16%
- 2010: 17.4%
- 2011: 19.4%
- 2012: 20%
- 2013: 21.2%
- 2014: 24.4%
- 2015: 26.6%
- 2016: 27.3%
TB diagnostic algorithm

1. Detection of *M. tuberculosis* in diagnostic sample collected before treatment initiation (smear microscopy, molecular-genethic method, culture on 2 samples)

2. Species differentiation between tuberculosis and non-tuberculosis mycobacteria

3. Drug susceptibility testing

Detection of MTB resistance markers using molecular methods

1st line DST

2nd line DST
Development of national reference laboratories network

On 27 April 2015 three bacteriology laboratories in federal research institutes were awarded the status of centres of excellence of the WHO TB supranational reference laboratories network.
Russian technologies for molecular-genethic diagnostics of tuberculosis

2005

Hydrogel biochip technology

Engelhardt Molecular Biology Institute

Tuberculosis and Pulmonology Research Institute — MoH Russia national medical research centre of tuberculosis, pulmonology and infectious diseases

2008

Multiplex allele-specific RT-PCR

Hydrogel biochip technology
RT-PCR, AmpliTube technology

- AMPLITUBE-DIFFERENTIATION
  - Differentiation of species within *M. tuberculosis* complex

- Clinical sample - DNA
  - Detection and quantification of *M. tuberculosis* complex
    - AMPLITUBE-RT
    - AMPLITUBE-Beijing (Genotyping)
    - AMPLITUBE-MDR-RT
    - AMPLITUBE-FQ-RT

- Non-tuberculosis mycobacteria testing
  - AMPLITUBE-RT+NTM

- Drug susceptibility testing
  - MDR (1\textsuperscript{st} line): Rifampicin, Isoniazid
  - (2\textsuperscript{nd} line): fluoroquinolones

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RT-PCR AmpliTube technology: MTB quantification

- Uses 2 *M. tuberculosis complex* specific gene sequences, which increases reliability

- Single-copy gene *regX3* of *M. tuberculosis complex* allows precise quantification of MTB cells in a sample

- Concurrent quantification of multi-copy insertion sequence *IS6110* and *regX3* gene already at this stage provides initial data on the abundance of *IS6110* and, therefore, the strain of *M. tuberculosis complex*

Ct *IS6110* (FAM) - Ct *regX3* (ROX) = 3-5 cycles

Ct *IS6110* (FAM) = Ct *regX3* (ROX)
AmpliTube, RT-PCR technology

MTB susceptibility test is based on new molecular technology is **multi-concurrent allele-specific RT-PCR**

**Detection of mutations:** 11 RMP mutations, 6 INH mutations, 6 FQ mutations

A: Reaction mix

B: Mutation

C: No mutation
RT-PCR AmpliTube, assay stages. Cost of test

Time to results – 2 days, cost of one test– 1000 RUR (~17 USD)

Vakhrusheva, "Tuberculosis and lung diseases", 2017
Diagnostic characteristics of AmpliTube-RV, AmpliTube-MDR and AmpliTube-FQ kits

MTB detection:
• Specificity 99-100%);
• Sensitivity 50-100 cells/mL

<table>
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Vladimirsky, "Tuberculosis and lung diseases", 2008
BIOCHIP technology

MDR (1st line), isoniazid, rifampicin

XDR (2nd line), fluoroquinolones

DST to rifampicin, isoniazid, fluoroquinolones, amikacin, kanamycin, capreomycin and ethambutol (120 genetic determinants analysed).

Genotyping

MTB differentiation:
M. bovis BCG,
MTB genotyping

Differentiation of tuberculosis and non-tuberculosis mycobacteria

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BIOCHIP technology

Assay is based on hydrogel biochip technology developed by Molecular Biology Institute, Russian Academy of Sciences. The fundamental distinction of the assay lies in the immobilisation (fixation) of molecular probes in three-dimensional semi-spherical elements affixed to a flat plate. Low density hydrogel-based biochips have a significant potential in identification of clinically significant mutations and single-nucleotide polymorphism in human, viral and bacterial genomes.

Production is certified under ISO 13485-2012 (TUV Rheinland Group, Germany)

Output of up to several thousand biochips a day
Stages of BIOCHIP assay

1. Sample preparation and DNA extraction

2. Multiplex PCR with concurrent fluorescent marking

3. Hybridisation on biochip

4. Recording and interpretation of results using biochip analyser

Time to results is 3 days, cost of one test is 3000 RUR (~53 USD)

Vakhrusheva, "Tuberculosis and lung diseases", 2017
<table>
<thead>
<tr>
<th><strong>TB-TEST – new generation reagent kit, 2017</strong></th>
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<tbody>
<tr>
<td><strong>Rifampicin</strong> (rpoB)</td>
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<tr>
<td><strong>Kanamycin, amikacin</strong></td>
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<td><strong>Capreomycin</strong> (rrs, eis)</td>
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<td><strong>Isoniazid</strong> (katG, inhA, ahpC)</td>
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<td><strong>Ethambutol</strong> (embB)</td>
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<td><strong>Genotyping</strong> (Beijing, LAM, Ural...)</td>
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DNA of tuberculosis complex mycobacteria is detected (IS6110 fragment).

Mutations responsible for rifampicin resistance are not detected (rpoB).

Mutations responsible for fluoroquinolones resistance are not detected (gyrB, gyrA).

Mutations responsible for isoniazid resistance are not detected (inhA, katG, ahpC).

Mutations responsible for amikacin, kanamycin and capreomycin resistance are not detected (rrs, eis).

Mutations responsible for ethambutol resistance are not detected (embB).

The strain belongs to Euro-American lineage. Genotype is not identified.

DNA of tuberculosis complex mycobacteria is detected (IS6110 fragment).

Mutation in rpoB gene causing rifampicin resistance.

Mutation type S531L

Mutation in katG gene causing isoniazid resistance.

Mutation type S315T(1)

Mutation in gyrA gene causing fluoroquinolones resistance.

Mutation type D94N S95T

Mutation in rrs gene causing amikacin, kanamycin and capreomycin resistance.

Mutation type a1401g

Mutation in embB gene causing ethambutol resistance.

Mutation type G406D

The strain does NOT belong to Euro-American lineage. Genotype: Beijing, variant B0/W148
Sensitivity of different methods of tuberculosis identification

Zimenkov et al., Journal of Antimicrobial Chemotherapy, 2016
Distribution of MTB genotypes in drug-resistant isolates from the North-Western district. Diagnostic characteristics of TB-TEST kit

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<td>Ethambutol</td>
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(N = 264)

Zimenkov et al., Journal of Antimicrobial Chemotherapy, 2016
Users of Russian test systems published over 150 articles in peer-reviewed Russian and foreign journals on the efficiency of early diagnosis of drug-resistant tuberculosis using Russian test systems.

**Dynamics of conversion**

- DST using TB-Biochip, if MDR is detected – change to 2\textsuperscript{nd} line and additional drugs
- DST using solid media culture, initiation 1 category regimen with subsequent correction based on DST results

**Dynamics of closure of lung cavities**

Gryadunov et al., 2011, *Expert Review of Molecular Diagnostics*

I.A. Vasilyeva, 2012 *Vestnik Rossiiskoi Akademii Meditsinskikh Nauk*
I. DIAGNOSIS

- **Diagnostic sample***
- **Sediment (NACL-NaOH)**
- **Complex examination: microscopy, RT-PCR AmpliTube-RT, MGIT, LJ**

II. MTB DST

**Result: positive and over 10 cells**

- **AmpliTube MDR-RT or BIOCHIP: H, R res mutations**
  - **Result:**
    - **DR H, DS R**
    - **DR H, R**
    - **DS H, R**

- **BIOCHIP TB-TEST: H, R, E, Fq, AG/CP**
  - **Result:**
    - **DR H**
    - **DS R, Fq, AG/CP**

- **If MGIT+, 1st line DST:**
  - **S, H, R, E**

**MGIT result:**

- **DR R, H**
- **DS H, DS R**
- **Fq**
- **DR Fq**

**MGIT result:**

- **Res: MGIT+, MGIT+, 2nd line DST:**
  - **Am/Km, Cm, Lfx, Mfx1, Mfx2, Pto/Eto, Pas, Z, Lzd***

**2nd line DST on MGIT:**

- **Of, Pto/Eto, Z, Am/Km, Cm**

**MGIT result:**

- **DR H, DS R**
- **Rезультат: устойчивость MGIT H+, R-, E+**

**DST using absolute concentrations method to Cs**

**Bronchoscopy to collect a sample and repeat examination***

- • 2 samples collected (sputum, pleural fluid, biopsy)
- • First sample: microscopy, molecular test, MGIT, solid media culture
- • Second sample: microscopy, solid media culture
- If previous sample is negative for MTB DNA, repeat molecular test and MGIT on second sample
- If both first and second sample are negative for MTB DNA, collect a third sample and repeat microscopy and molecular test
- *** If three samples are negative for MTB DNA, perform bronchoscopy to collect a sample and repeat microscopy, MGIT molecular test and solid media culture
Zsuzsanna Jakab, Director of the World Health Organization's Regional Office for Europe, noted success of the Ministry of Health of Russia in fighting drug-resistant tuberculosis

«We are grateful for the leadership you displayed in controlling antimicrobial resistance revising national guidelines and recommendations on clinical management of M/XDR TB patients. Unique process of consultations between the Ministry of Health and the World Health Organization within the framework of high level TB working group and adoption of the Order no. 951 on 29 December 2014 were two milestones in this regard», – stressed Zsuzsanna Jakab.
The First WHO Global Ministerial Conference *Ending TB in the sustainable development era: A multisectoral response* aimed to accelerate implementation of the WHO End TB Strategy - with immediate action addressing gaps in access to care and the MDR-TB crisis - in order to reach the End TB targets set by the World Health Assembly and the United Nations (UN) Sustainable Development Goals (SDGs) through national and global commitments, deliverables and accountability.
Conference, its objectives, expected outcomes and draft declaration were actively being discussed for over a year by all stakeholders and countries and received support on over 20 major international fora and platforms, such as meetings of presidents and ministers of health, of BRICS countries, APEC, G-20, UN General Assembly meetings, WHA, WHO regional committees meetings, STAG-TB, Stop-TB Partnership co-ordination council, special meetings with partners, TB Caucus meeting, etc.
UN GENERAL ASSEMBLY DECISION TO CONVENE A HIGH-LEVEL MEETING ON THE FIGHT AGAINST TUBERCULOSIS IN 2018

- Declaration was adopted unanimously.
- 3 WHO member countries made statements in support of the Declaration: the USA, Canada and South Africa.
- The Declaration will be taken into consideration by the UNGA high-level meeting on the fight against tuberculosis in 2018.
Thank you for your attention!