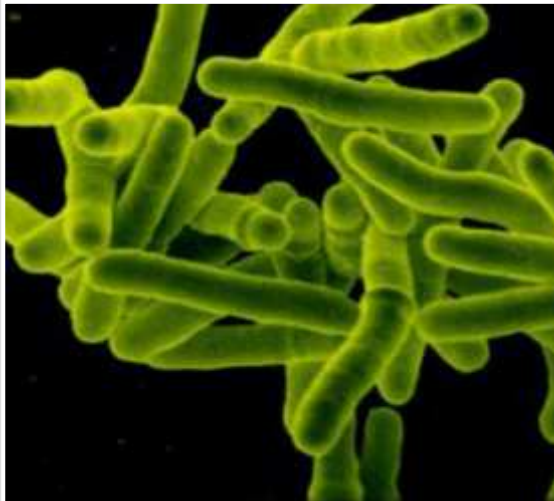


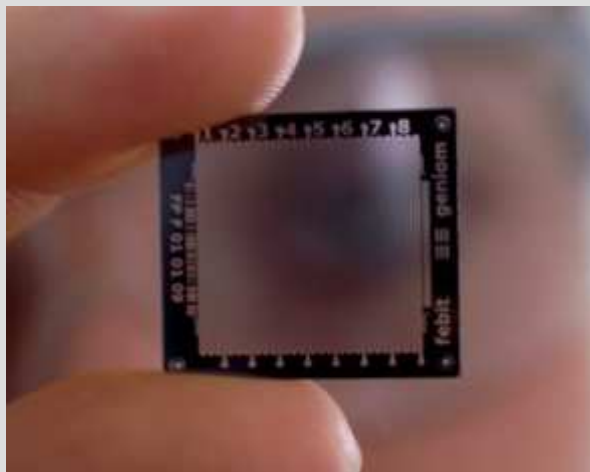
Enhelhardt Institute of Molecular Biology

Moscow, Russia

D.V. Zimenkov, D.A. Gryadunov, A.S. Zasedatelev, V.M. Mikhailovich



Biochips in tuberculosis diagnostics



Gel-based Biological Microchips (Biochip, Microarray)

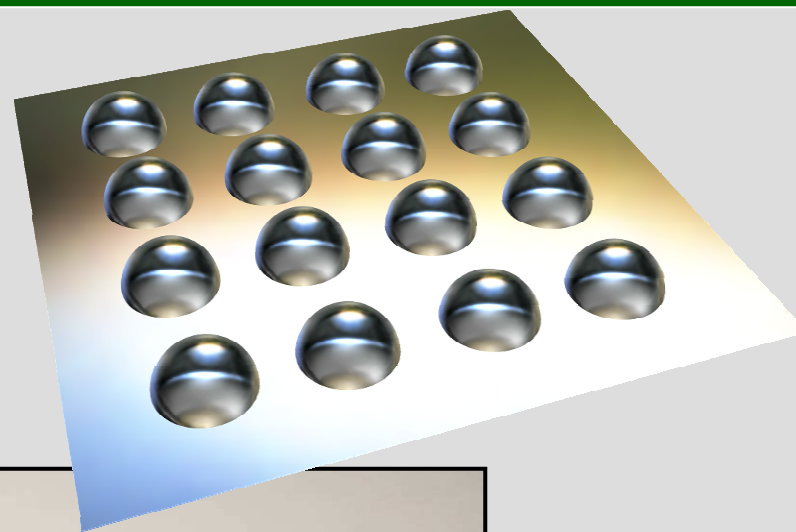
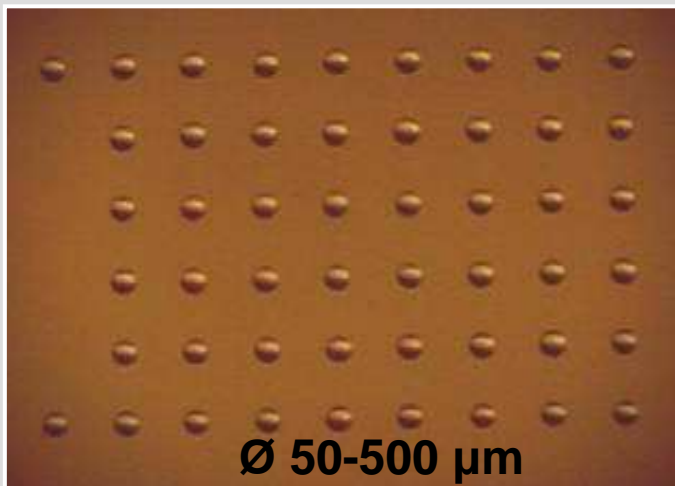


Engelhardt Institute of Molecular Biology

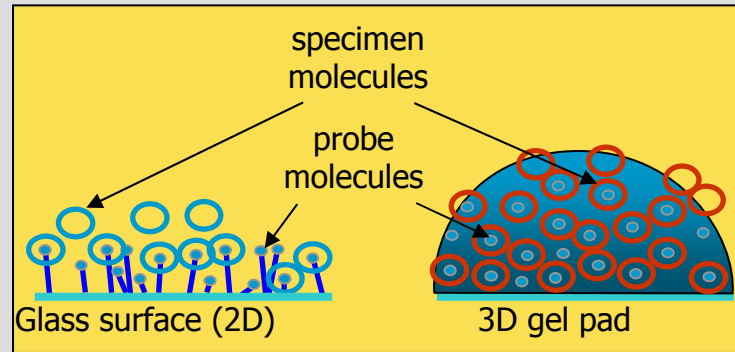


Andrei Mirzabekov
(1937-2003)

3-dimensional BIOCHIPS



Advantages of 3-dimensional BIOCHIPS



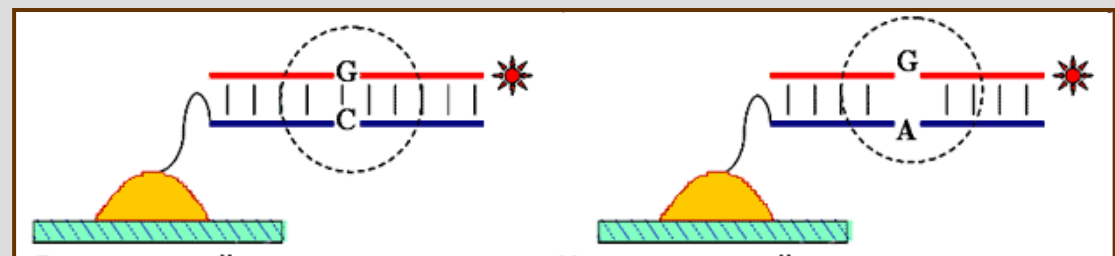
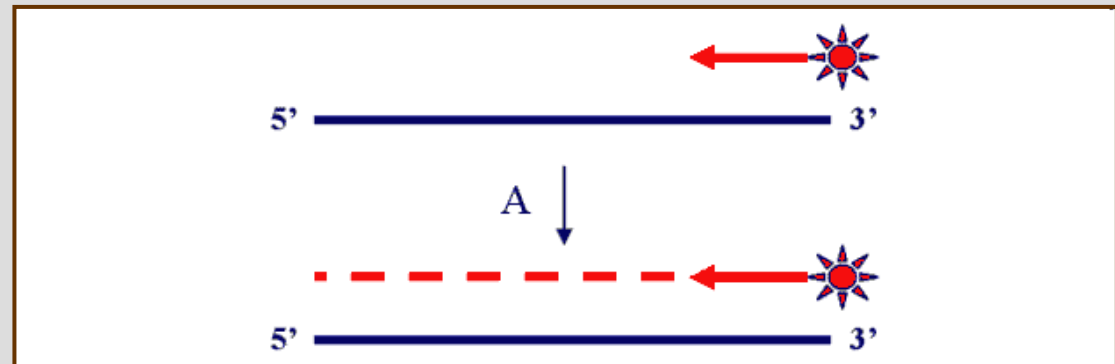
- Immobilization capacity is 1000x higher for 3D biochips which **increases analysis sensitivity** leading to very good discrimination level.
- There is **no contact** between immobilized probe molecules and support.
- Immobilized molecules retain their **biological activity**.
- Each cell acts as a nanotube wherein **any reaction** can be performed.

Principal Scheme of the Point Mutation Detection by Hybridization on Biochip

Fluorescent labeling of a tested DNA is performed using asymmetrical PCR with a labeled primer or labeled dNTPs

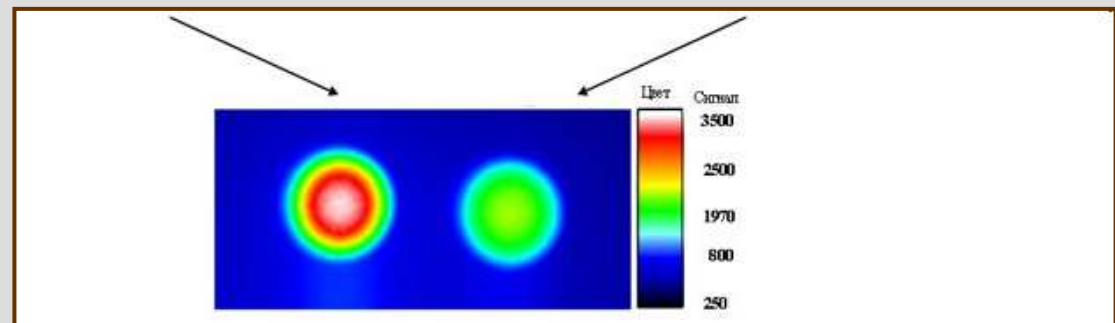
This product is hybridized to the on-chip immobilized probes specific to both wild-type and mutant DNA

The fluorescence intensities are compared to find out whether tested DNA bears a mutation or not



Perfect duplex

Imperfect duplex



BIOCHIP-IMB LLC

ISO and GMP certified

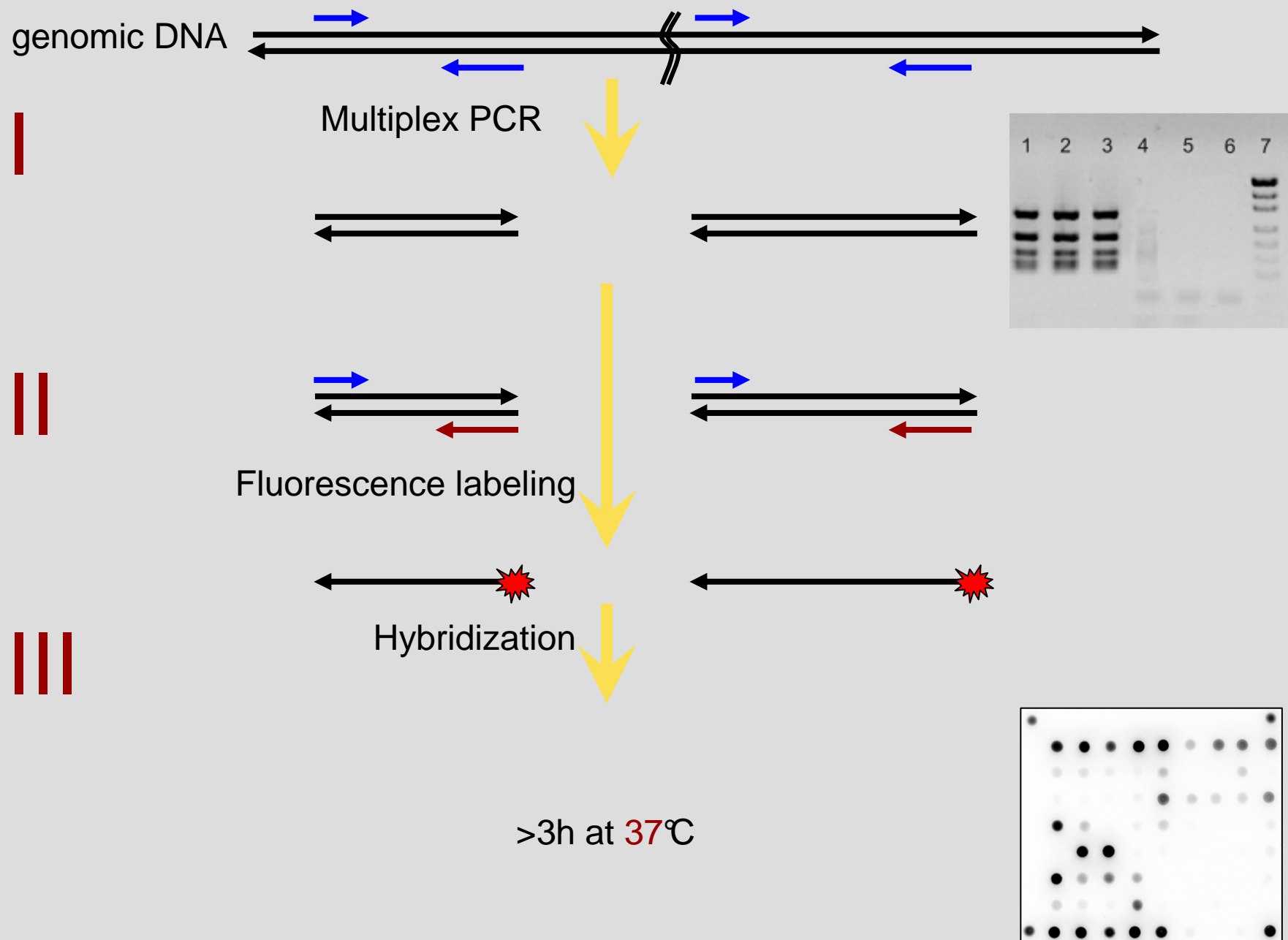
Several thousands of biochips could
be produced per day



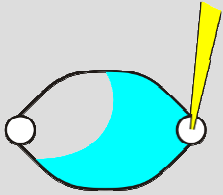
BIOCHIP-IMB LLC



Protocol of *M.tuberculosis* genome analysis

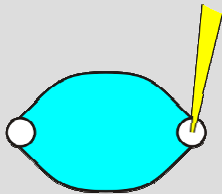


Hybridization procedure



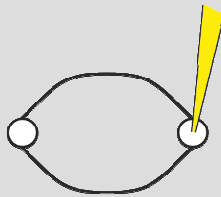
Place 20mkl of HB buffer in hybridization chamber

Take 10mkl of amplification mix after 2nd PCR...



... and add it to the same inlet

Incubate at 37°C for 3 and more hours (72 is OK)



Remove hybridization mix and wash twice with warm water

Remove chamber, wash with ethanol and water flow

Dry biochip

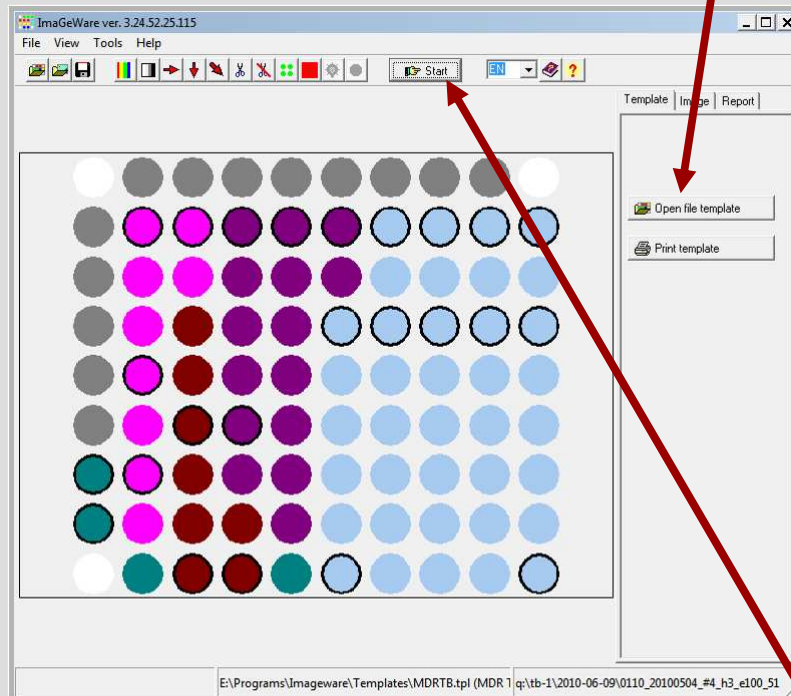
Biochip Analyzer



- Universal for any type of biochips produced by EIMB
- High speed and Sensitivity
- User-friendly software for medical personnel
- Certified in clinical trials

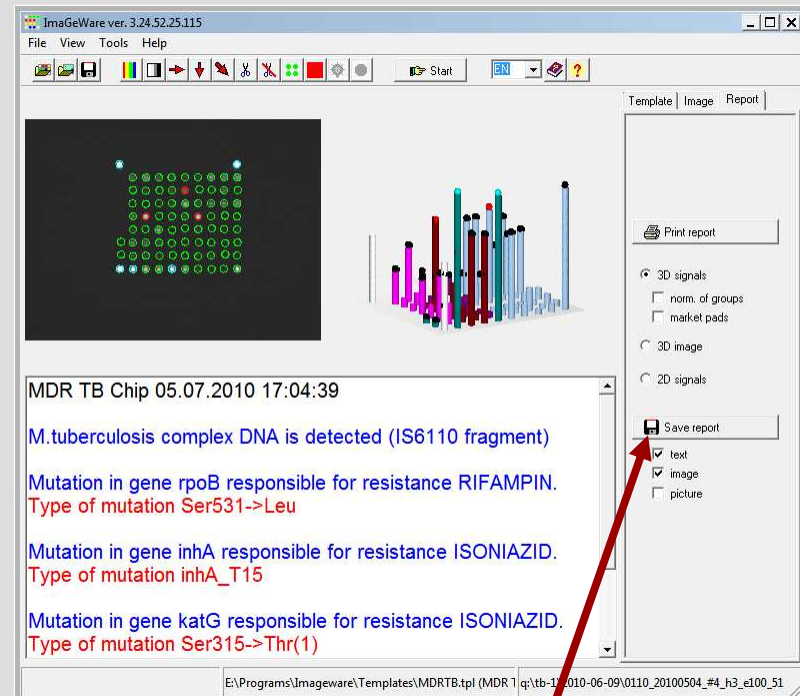
Analyzing biochips with ImageWare® soft

Select template (needed only when changing test system)



Place chip in Analyser

Press Start



Read and save Report

TB-Biochip (MDR)

ISTC Project #2019

Biochips application in TB diagnostics for fast discrimination and strain typing of multidrug-resistant tuberculosis in Russia

Lead Institute:

Engelhardt Institute of Molecular Biology, Russian Academy of Sciences

Sub-Institution:

State Research Center of Virology and Biotechnology Vector (SRC VB VECTOR)

Duration:

Main project: April 1st, 2001 – March 31, 2003 (24 Months)

Addendum I: April 1st, 2003 – March 31, 2004 (12 Months)

Addendum II: April 1st, 2004 – July 31, 2004 (5 Months)

Addendum III: August 1st, 2004 – December 31, 2004 (5 months)

TB-Biochip (MDR)

(M)									(M)
	ahpC w(C10)	ahpC w(G6)	Trp 328	Ser 315	Ser 315	Leu 533	Ser 522	Met 515	WT 507
	ahpC (T10)	ahpC (A6)	Gly 328	Thr 315 ₂	Thr 315 ₁	Pro 533	Leu 522	Ile 515	Del 507
	ahpC (A10)	inhA (G16)	Leu 328	Asn 315	Leu 511	Ser 531	His 526	His 526	Asp 516
	ahpC w(G9)	inhA (T15)	Cys 328	Ile 315	Pro 511	Leu 531	Tyr 526	Asp 526	Val 516
	ahpC (A9)	inhA w(C15)	Ile 335	Arg 315 ₁	Arg 511	Trp 531	Asn 526	Leu 526	Tyr 516
(O)	ahpC w(C12)	inhA (G8)	Val 335	Arg 315 ₂	Arg 512	Cys 531	Arg 526	Gln 526	Gly 516
(O)	ahpC (T12)	inhA (A8)	inhA (T24)	Gly 315	Thr 512	Gln 531	Pro 526	Cys 526	Glu 516
(M)	IS6110	inhA w(T8)	inhA w(G24)	IS6110	Ser 512	Gly 513	Lys 513	Leu 513	Gln 513

27 mut RIF(R)
20 mut INH(R)

Identify:

more than 95% RIF resistant forms of TB

more than 80% INH resistant forms of TB

during 24 hours.

4 years of use in Russian Federation and abroad, more than **40000** analyses

Certified by Russian Ministry of Health

Регистрационное удостоверение МЗ и СР РФ № ФС 03262004/0889-04

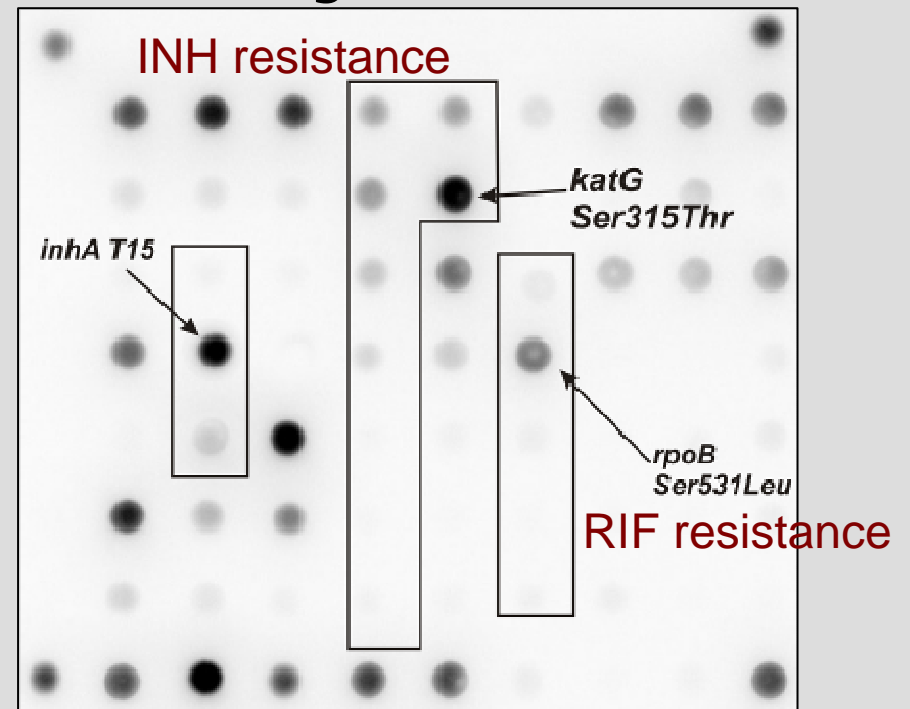
Identification of *M.tuberculosis* drug resistance

Wild Type



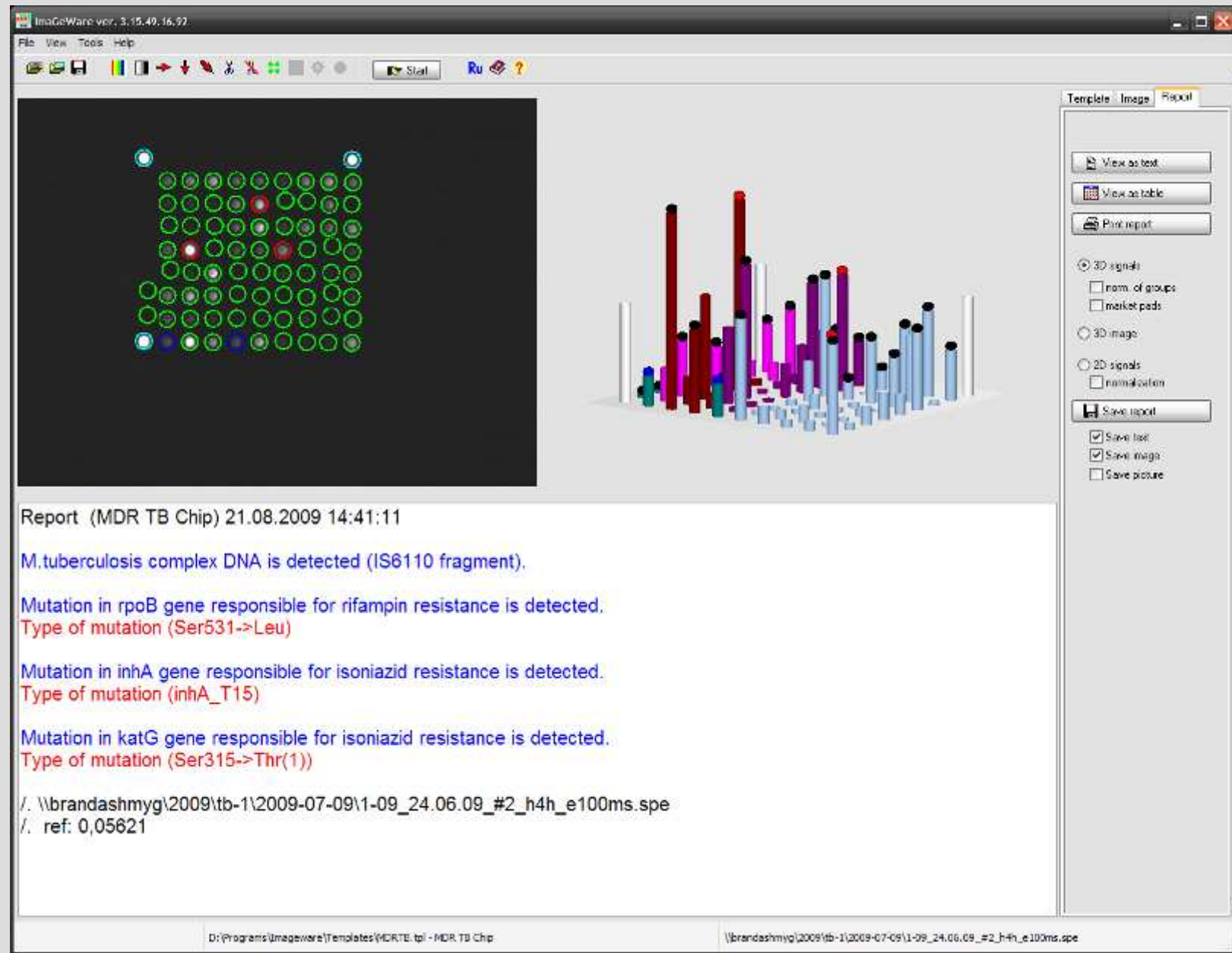
Routine therapy

Multidrug-resistant TB



Second line drugs should be used

Program window with analysis results



Sensitivity and specificity of TB-Biochip (MDR)

	Sensitivity, %	Specificity, %
RMP	90-97	86-94
INH	82-89	88-95

Poliakov AE, Safonova SG, Skotnikova OI. [Determination of multidrug resistance of *M. tuberculosis* by different methods]. *Probl Tuberk Bolezn Legk*, (6), 40-42 (2006).

Stepanshina V, Nizova A, Mayorova A et al. Susceptibility testing to rifampicin of *M. tuberculosis* clinical isolates by the methods of microbiology and molecular biology. *Biotechnology in Russia*, 1, 121-130 (2008).

Barilo V, Kuzmin A, Chernousova L. Accelerated determination of *Mycobacterium tuberculosis* susceptibility to essential antituberculous drugs, by using the Bacmec MGIT 960 system and TB-Biochips. *Probl Tuberk Bolezn Legk*, 86(11), 56-60 (2009).

Gayeva N, Zhilin O, Ivleva S, Ovchinnikova O. Experience in using TB-Biochip (MDR) test systems in the Lipetsk region. *Probl Tuberk Bolezn Legk*, 87(2), 57-59 (2010).

Isakova Zh T. [Practical value of the TB-biochip MDR test system in the rapid identification of multidrug-resistant *M. tuberculosis* strains]. *Klin Lab Diagn*, (2), 50-51 (2009).

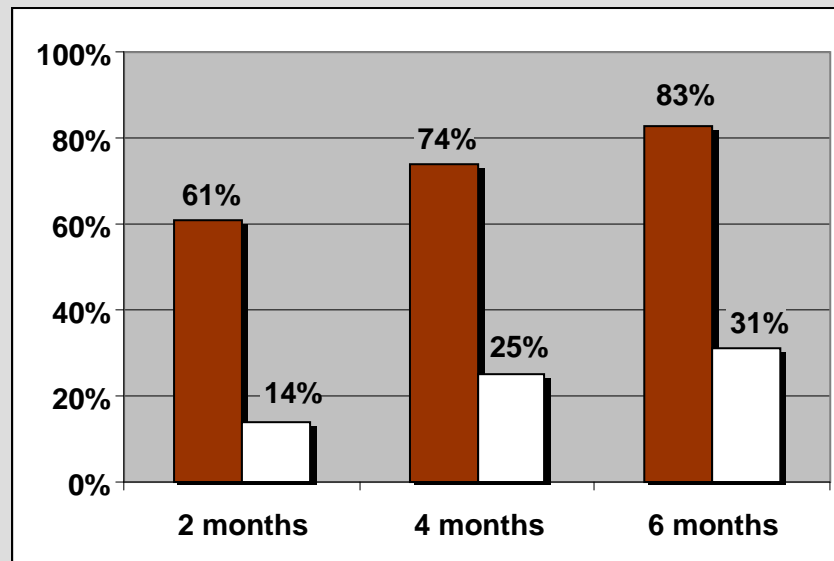
Manaenkova EV, Savin AA,
in press

		BACTEC MGIT 960		
		INH ^R	INH ^S	Total
TB-Biochip	INH ^R	100	9	109
	INH ^S	11	134	145
	Total	111	143	254
		BACTEC MGIT 960		
		RMP ^R	RMP ^S	Total
TB-Biochip	RMP ^R	95	3	98
	RMP ^S	3	153	156
	Total	98	156	254

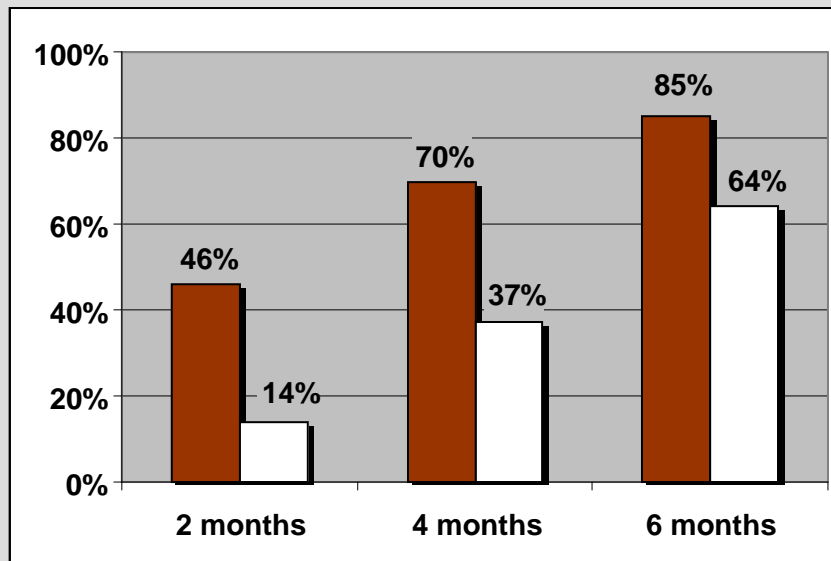
Clinical effects of TB-Biochip application

MDR tuberculosis

Bacterial conversion dynamics



Caverns healing dynamics



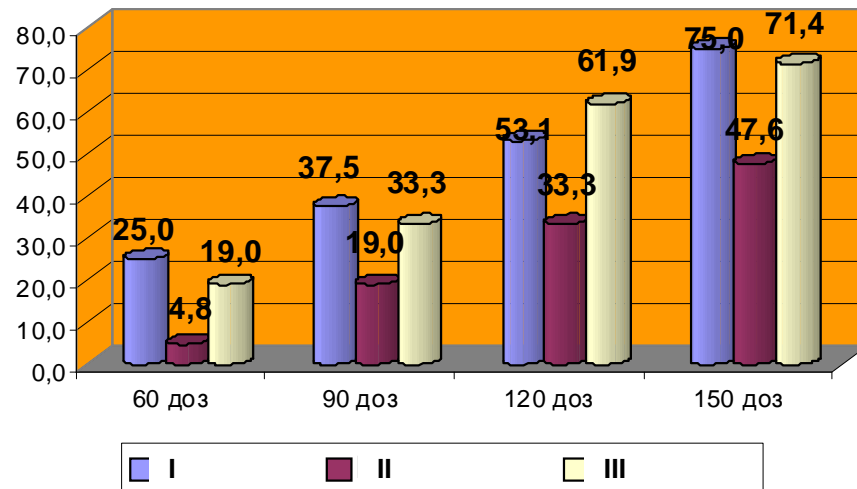
■ Therapy using TB-BIOCHIP results
□ Standard therapy

Central Tuberculosis Research Institute

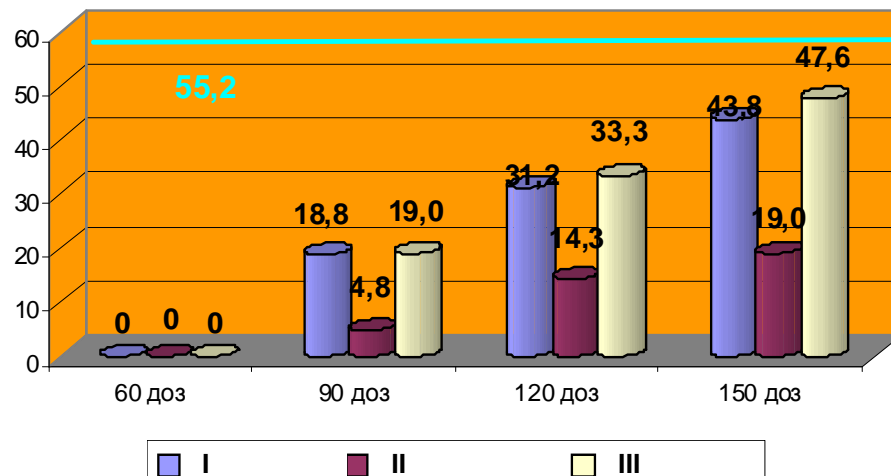
A. Kuz'min et al., "Efficiency of chemotherapy...", Probl Tuberk Bolezn Legk. 2006;(8):17-23

Clinical effects of TB-Biochip application

Bacterial conversion dynamics



Caverns healing dynamics



I – Resistance to 1-2 drugs of 1st line
(H,R,E,S,HS,HE)
n=32

Treating: 2-3 weeks H R Z E (S) → results of TB drug susceptibility → changing of 2 drugs to Pr+Fq

II – MDR (HR,HRS, HRE)
n=21

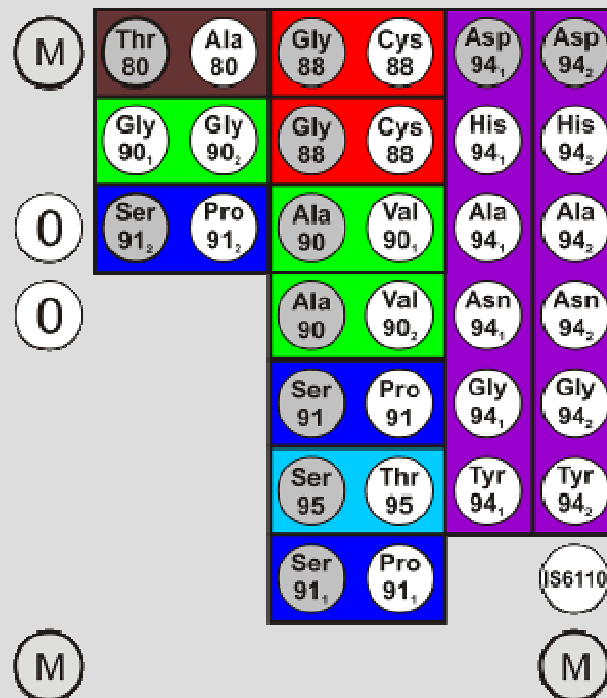
Curing: 2-3 weeks H R Z E (S) → results of TB drug susceptibility → Z E Pr Fq Cs (Pas)

III – MDR (HR-obtained by TB-Biochip)
n=21

Curing: results of TB drug susceptibility **by TB-Biochip** → Z E Pr Fq Cs (Pas)

TB-Biochip (FQ)

TB-Biochip (FQ)



Identifies:

More than 80% of FQ-resistant strains

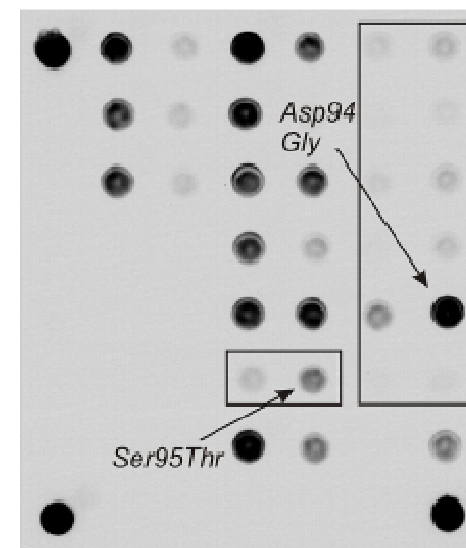
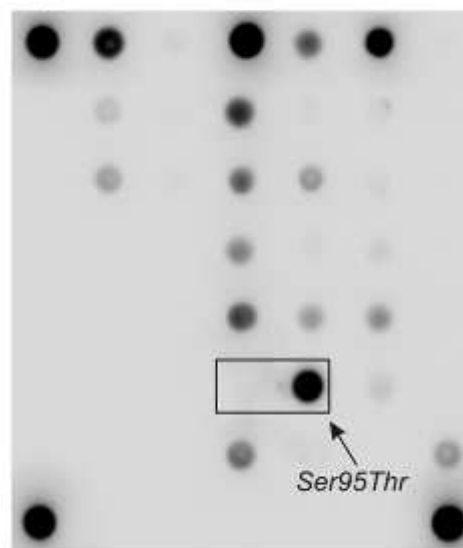
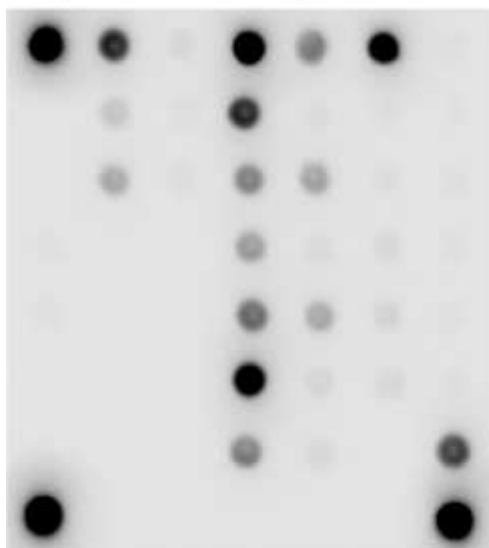
More than 3000 analyses

Certified by Russian Ministry of Health

Регистрационное удостоверение МЗ и СР РФ № ФС 01012006/3527-06

Identification of FQ-resistance of MBT

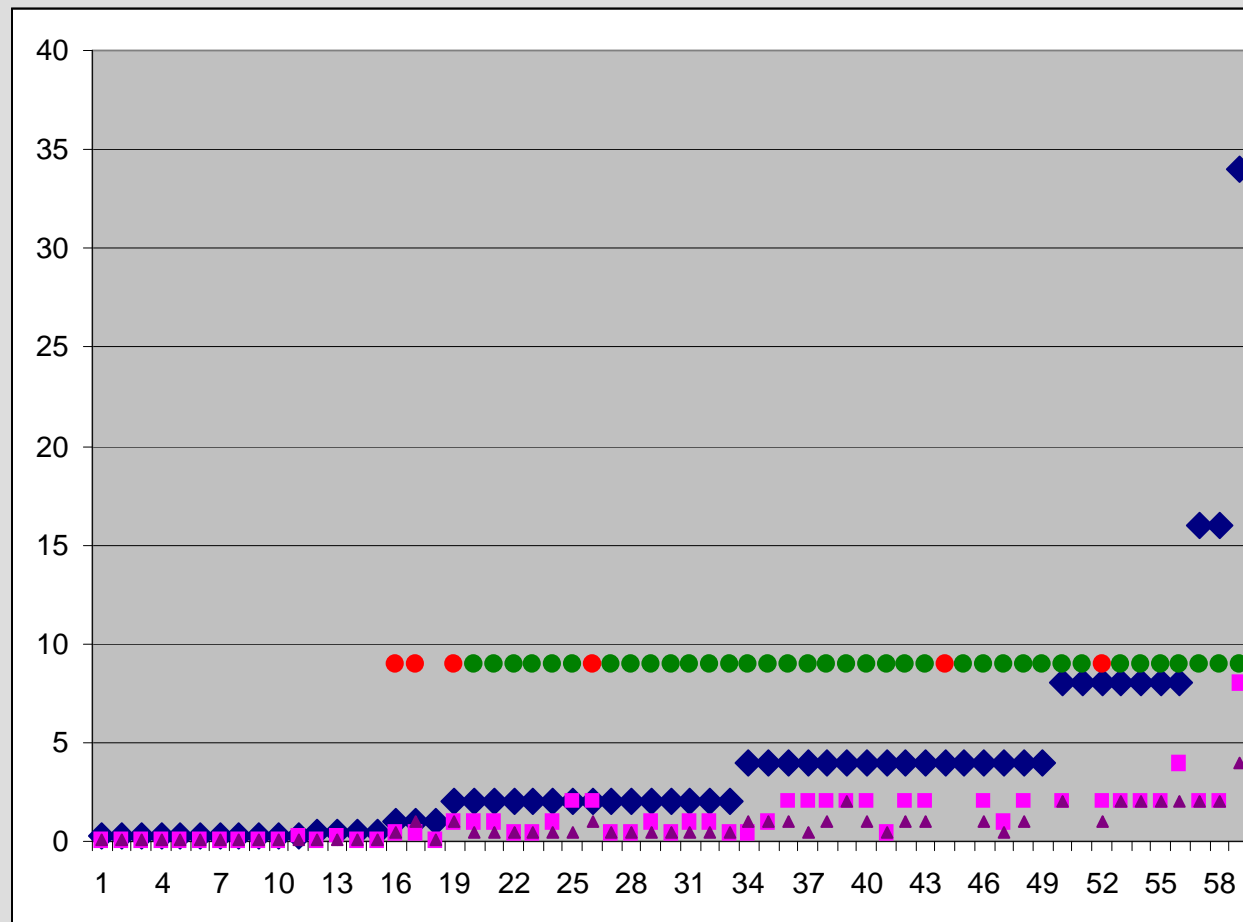
FQ-resistance



Naturally occurring
polymorphism

XDR-Biochip

- ◆ OFX MIC, mkg/ml
- MOX MIC, mkg/ml
- ▲ GATI MIC, mkg/ml

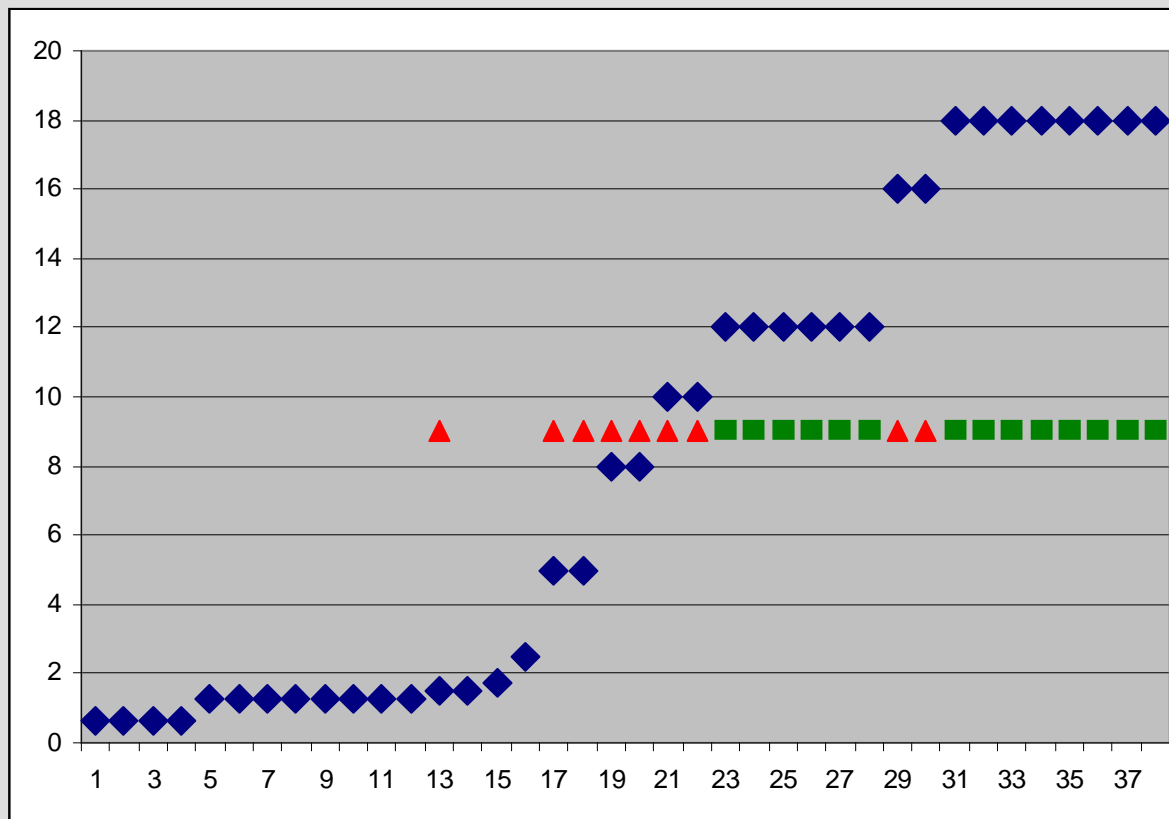


- mutations detected by TB-Biochip (FQ)
- mutations detected by XDR-Biochip

*EIMB RAS / Moscow Scientific-and-Practical Center for Tuberculosis Control
unpublished*

XDR-Biochip

◆ KAN MIC,
mkg/ml

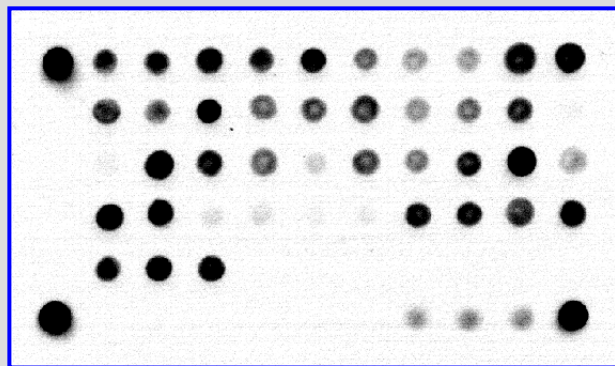
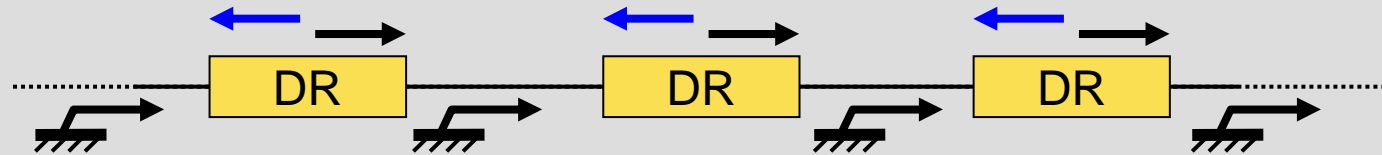


■ mutations detected by XDR-Biochip version 1

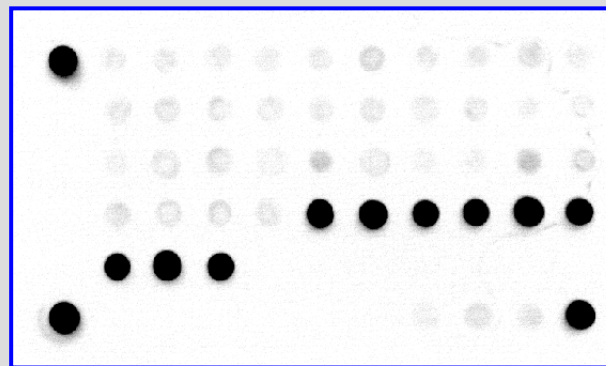
▲ additional mutations detected by XDR-Biochip version 2

Spoligo-Biochip

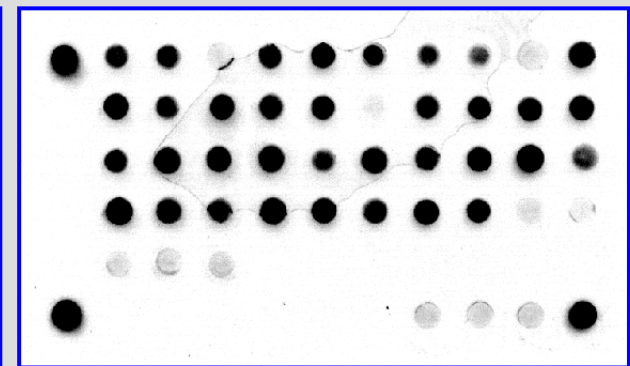
Analysis of DR-region of MTB complex (Spoligotyping)



M. tuberculosis H37Rv



M. tuberculosis Beijing



M. bovis

Analysis of DR-region of MTB complex (Spoligotyping)

Beijing	46
T1	5
NEW	4
H3	2
U (likely H3)	2
LAM9	2
H4	1
T2	1
T5_Rus1	1
X1	1
88	1

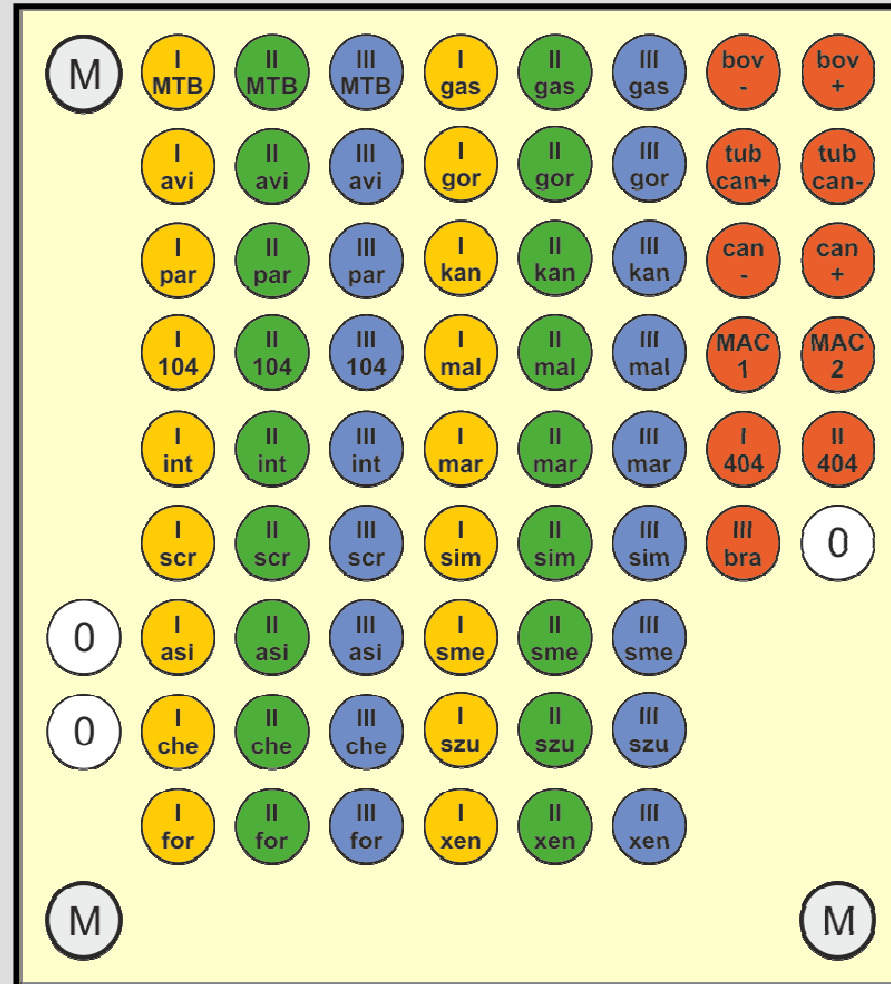
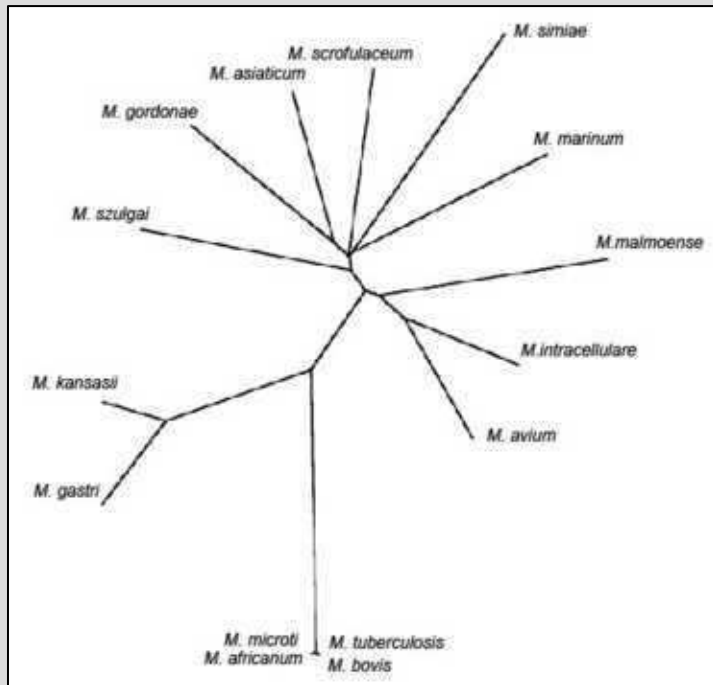
70%

Total

66

MYCO-Biochip

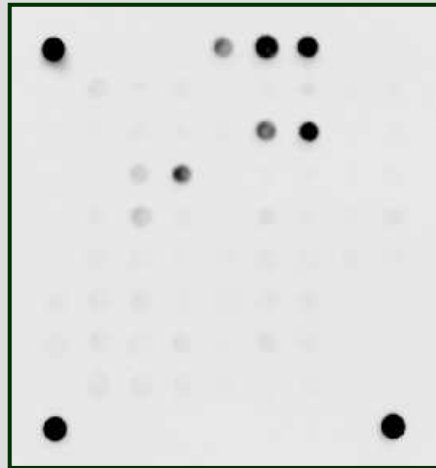
Biochip for *Mycobacteria* species differentiation



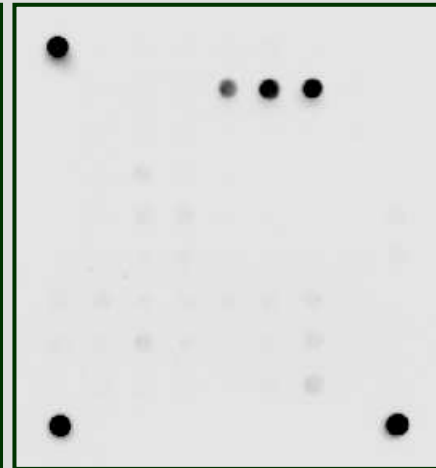
Biochip for *Mycobacteria* species differentiation



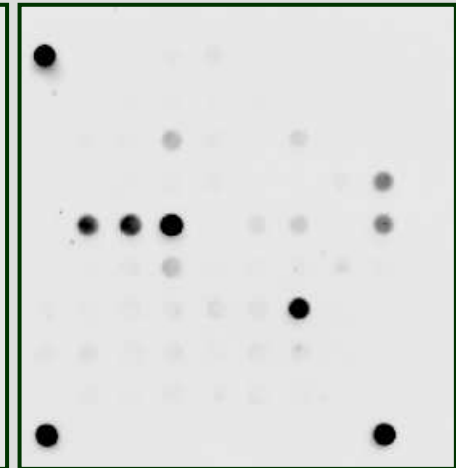
M.chelona



M. gastr



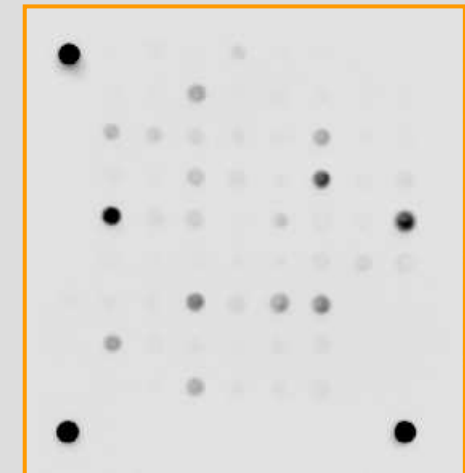
M. gordonae



M.intracellulare

tuberculosis, avium, paratuberculosis, avium 104, intracellulare, scrofulaceum, asiaticum, chelonae, fortuitum, gastri, gordonae, kansasii, malmoense, marinum, simiae, smegmatis, szulgae, xenopi

kumamotonense(terrae), septicum, peregrinum, phlei, flavescence, mantenii(MAC), GR-2002-583, sp. CCUG 52297



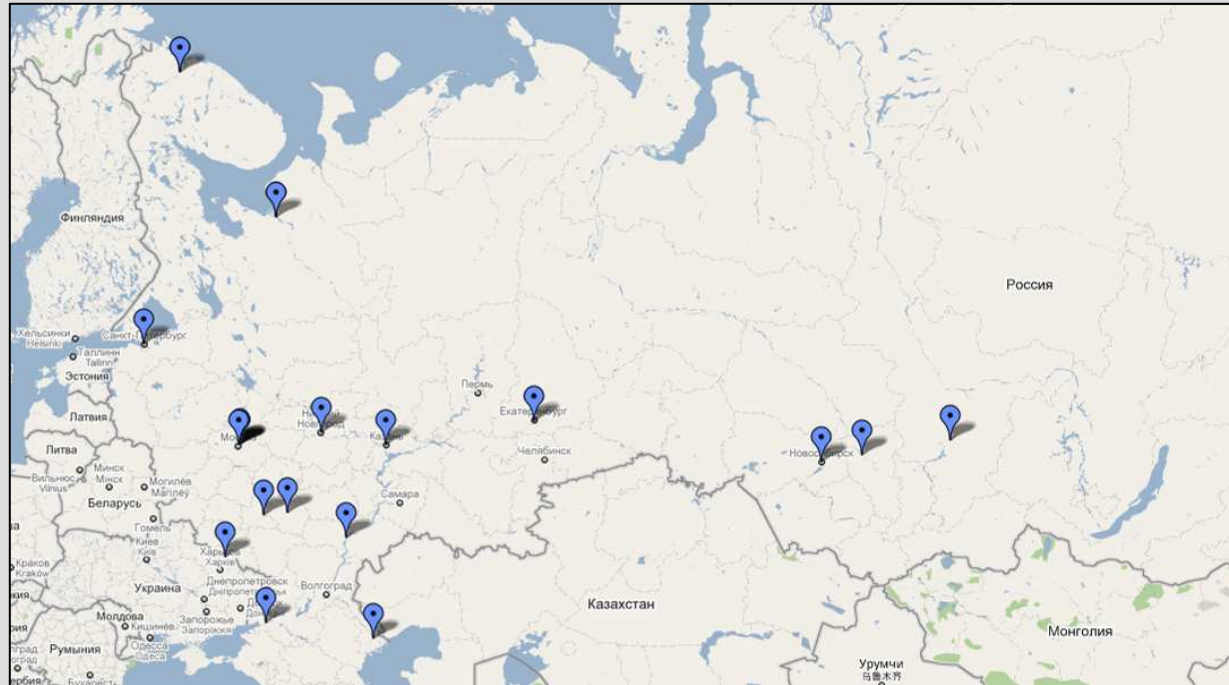
M.phle

Biochip for *Mycobacteria* species differentiation

<i>M.avium</i>	67
<i>M.fortuitum</i>	52
<i>M.xenopi</i>	43
<i>M.kansasii</i>	27
<i>M.intracellulare</i>	15
<i>M.gordonae</i>	13
<i>M.abscessus</i>	8
<i>M.bovis</i>	2
<i>M.chelonae</i>	1
<i>M.flavescens</i>	1
<i>M.kumamotonense</i>	1
<i>M.mantonii</i>	1
<i>M.phlei</i>	1
<i>M.septicum</i>	1
<i>M.peregrinum</i>	1
<i>M.GR-2002-583</i>	1
<i>M.sp. CCUG 52297</i>	1
<i>M.interjectum</i>	1
<i>M.intermedium</i>	1

EIMB RAS / Moscow Scientific-and-Practical Center for Tuberculosis Control
unpublished

Labs using TB-Biochip



More than 20 centers in Russian Federation, Kyrgyzstan and Azerbaijan

THANK YOU FOR YOUR ATTENTION !