

ADVANCE LABORATORY INVESTIGATIONS OF MDR/XDR-TB

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MINISTERS MEETING TO ENDTB IN MOSCOW, 16-17 NOVEMBER 2017

75 Ministers from 100 countries went to Moscow to declare
increasing investment for End TB by 2030



PRESIDENT PUTIN OPENING SPEECH



Notably, the death rate from TB in Russia has declined by more than 66 percent, and the incidence rate by 37 percent over the past eight years. During the specified period, the incidence has been decreasing by an average of 1.5 percent internationally, whereas in Russia the rate of decline was 2.8 percent.

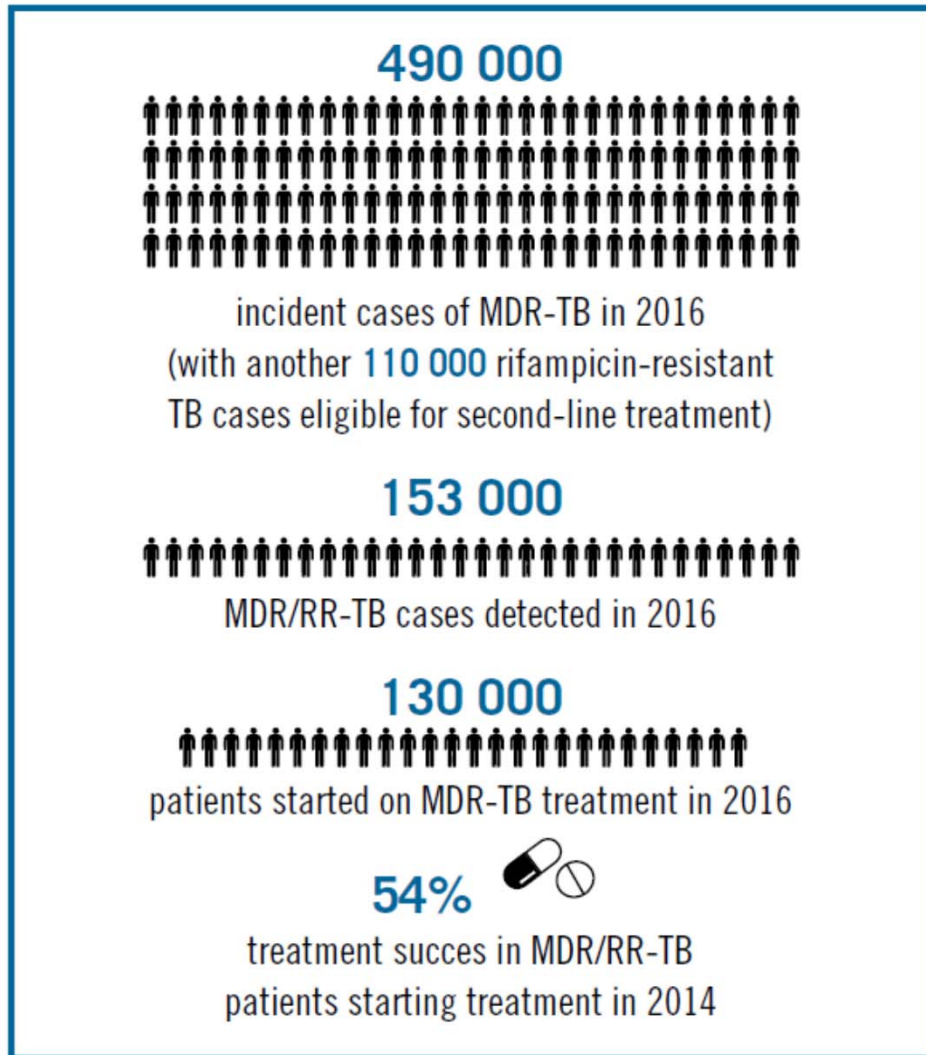
However, in order to achieve a radical change in the fight against this disease, of course, **new approaches are needed, both at the national and international levels, as well as the joint work of governmental agencies, public and professional organisations.**

Another important success factor is to **step up scientific tuberculosis research and develop effective diagnostic tools, vaccines and medicines, including those aimed at treating resistant forms of tuberculosis.** In this regard, I believe that the initiative of the BRICS countries to create a network to study tuberculosis is very important. Specialists are already working on this project.

<http://en.kremlin.ru/events/president/news/56107>

Figure 1:

The global MDR-TB response, 2016





Application of new technology for EndTB strategy in Thailand

Target

171/100,000
incidence in 2014

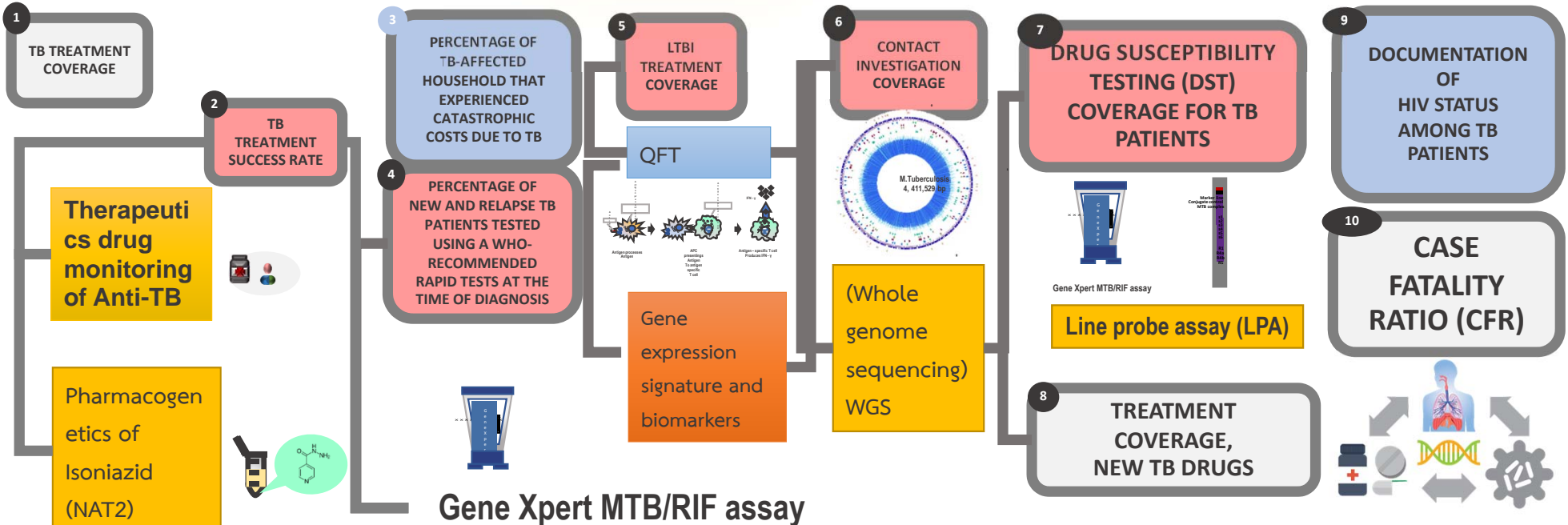


Reduction
12.5 % per annum



88/100,000
In 2021

TEN Global PRIORITY INDICATORS (GPIs)



UNIVERSAL (100%) DST IS MANDATORY FOR ENDTB

DST- WHO endorsed technology

- Liquid culture and DST
- RPOCT (GeneXpert)
- Line Probe Assay

**DRUG SUSCEPTIBILITY
TESTING (DST)
COVERAGE FOR TB
PATIENTS**

News Home » India

Delay in diagnosis worsens drug-resistant tuberculosis in country



The fight against tuberculosis just got fiercer. The Centre is ready to announce universal drug susceptibility testing (DST) in Mumbai as a pilot project for the rest of the country, said Dr Sunil Khaparde, deputy director



CULTURE FOR TB IS CHALLENGING

Need High biosafety level

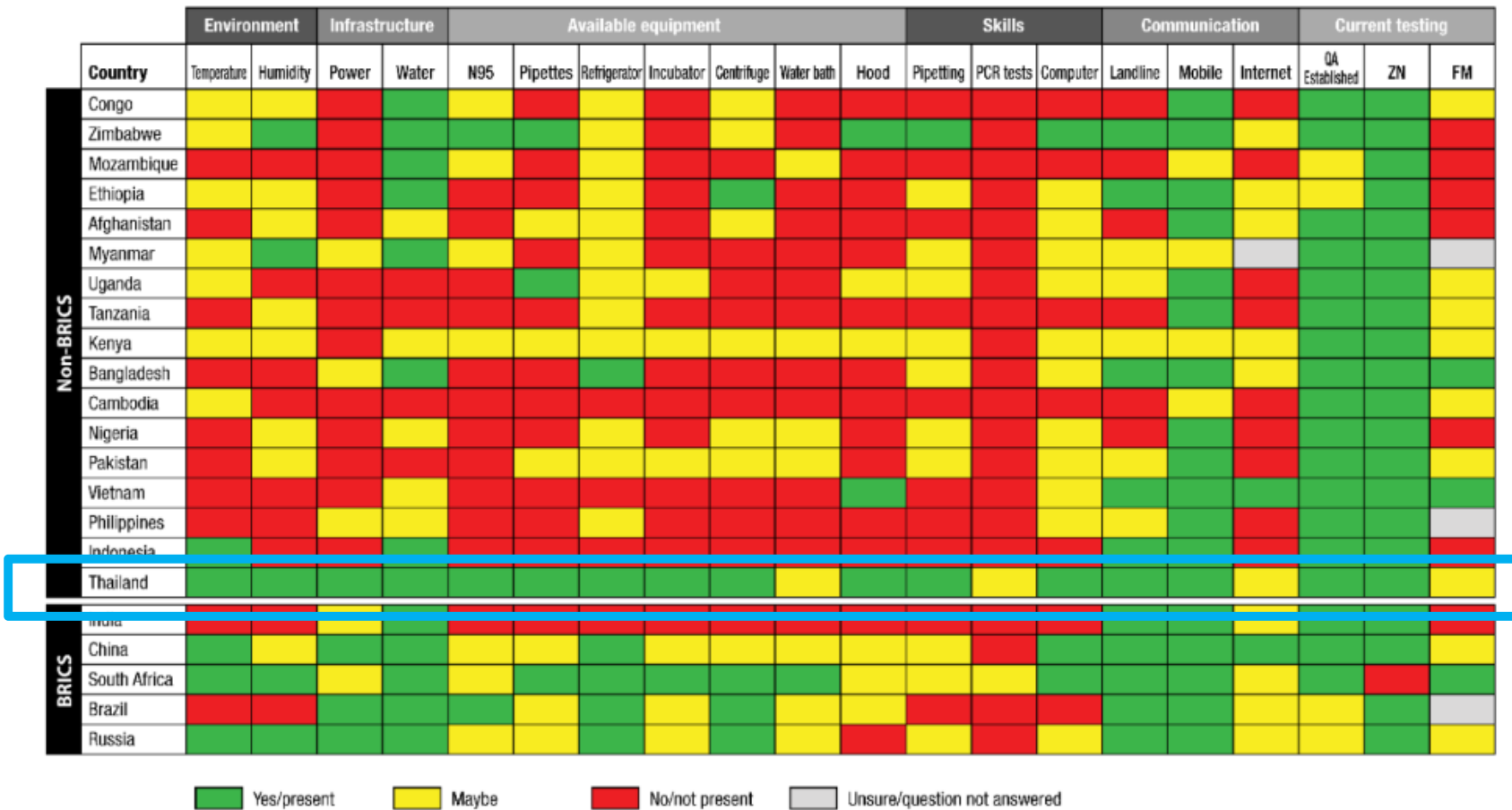
Drug susceptibility testing is difficult to mastered, need high level laboratory skills

Usually, only few laboratories can achieved the quality that acceptable for clinical usages

Only few drugs can do drug susceptibility testing with clinically meaningful results (First line anti-tuberculosis and few second line anti-tuberculosis)



Figure 34. Heat map showing characteristics of peripheral microscopy centres in 22 high-TB burden countries



RPOCT FOR TB AND RIFAMPICIN RESISTANCE (GENEXPERT)

1

Pour Sample Reagent into sample tube. Incubate for 15 minutes at room temperature. (Acceptable sample types: unprocessed sputum or sediment from concentrated specimen.)



2

Pipette diluted sample into cartridge.

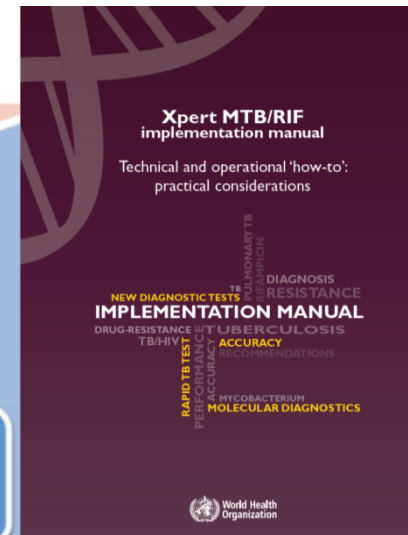
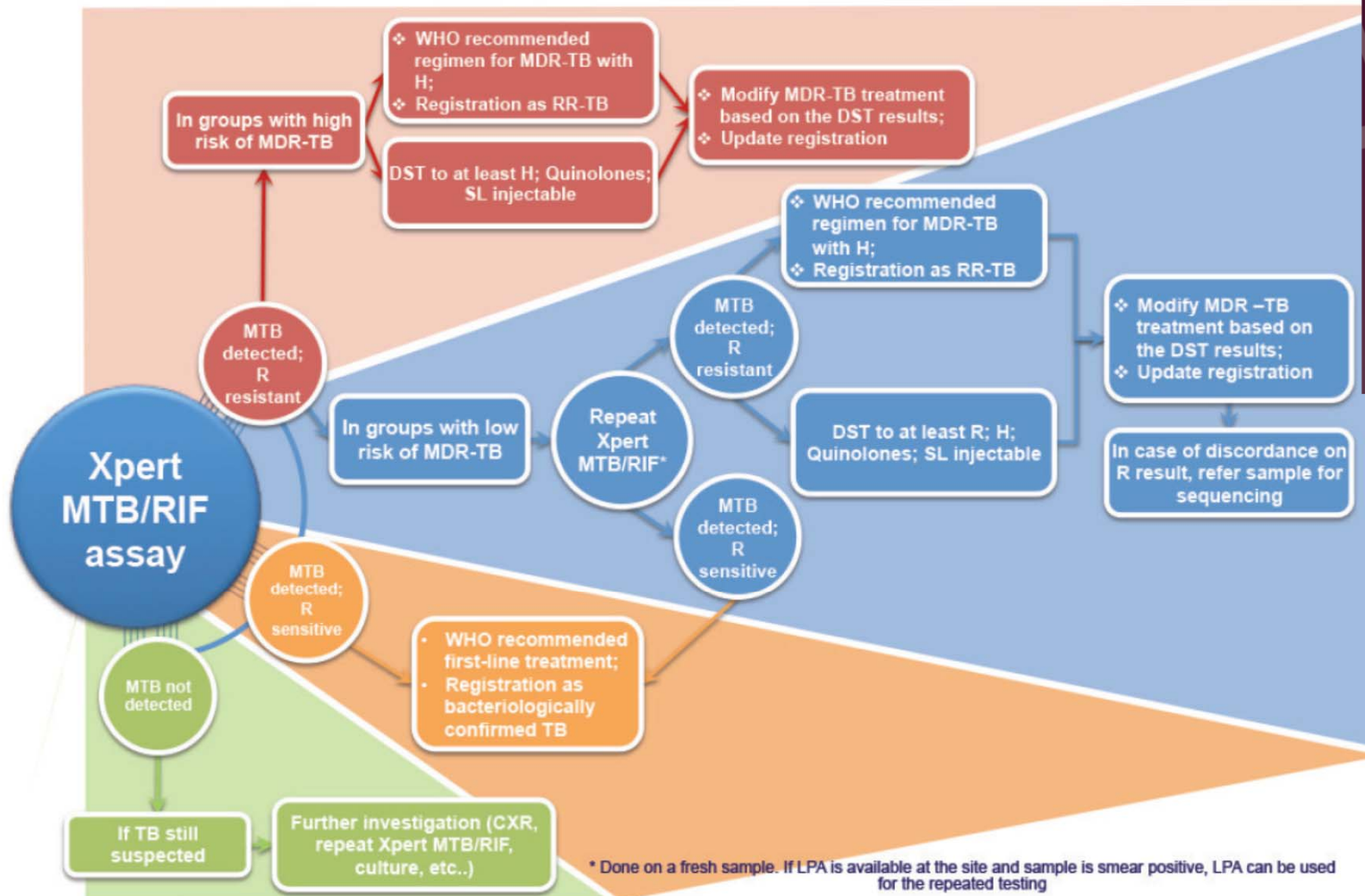


3

Insert Cartridge and start assay.



Figure 1. Interpreting results from Xpert MTB/RIF tests

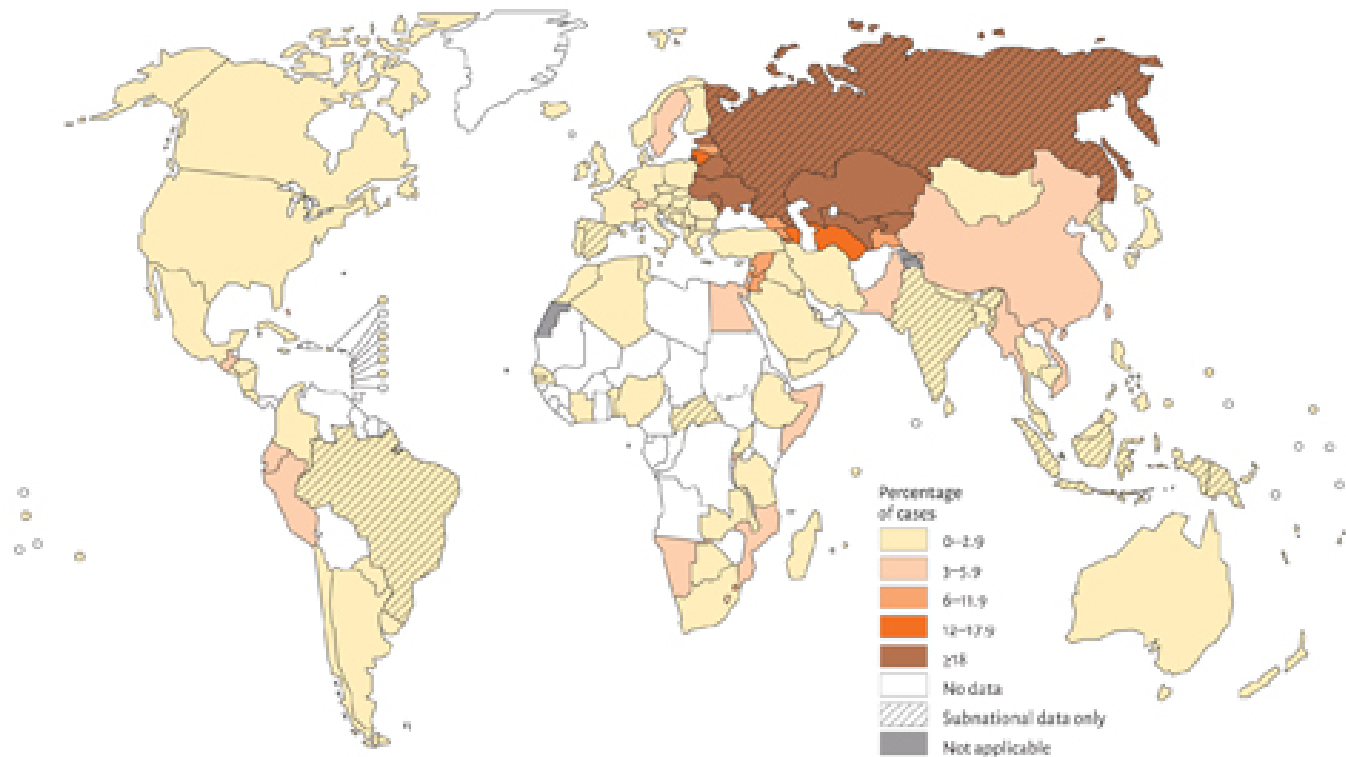


Xpert MTB/RIF implementation manual Technical and operational 'how-to': practical considerations (WHO, 2012)

COUNTRY	DIAGNOSIS			
	Xpert MTB/RIF is the initial TB diagnostic test for adults and children being investigated for TB	TB-LAM is used to diagnose TB in PLWHA with CD4 \leq 100 μ L or seriously ill	First-line DST (rifampicin and isoniazid) is done for all RR-TB cases or for people at risk of DR-TB	Second-line DST (fluoroquinolones & second-line injectable agents) is done for all DR-TB cases
Afghanistan	●	●	●	●
Armenia	● ^a	●	●	●
Bangladesh	●	●	●	●
Belarus	● ^b	●	●	●
Brazil	●	●	●	●
Cambodia	●	●	●	● ^c
CAR	●	⊗	●	?
China	●	●	●	●
DRC	●	●	●	● ^c
Ethiopia	●	●	●	●
Georgia	●	●	●	●
India	●	●	●	●
Indonesia	●	●	?	●
Kazakhstan	●	●	●	●
Kenya	●	●	●	●
Kyrgyzstan	● ^d	●	●	●
Mozambique	●	●	●	● ^c
Myanmar	●	●	● ^e	● ^f
Nigeria	●	●	●	⊗
Pakistan	●	●	●	?
PNG	●	●	●	●
Philippines	●	●	●	●
Russian Fed.	● ^g	●	●	●
South Africa	●	●	●	●
Swaziland	●	●	●	●
Tajikistan	●	●	●	●
Ukraine	●	●	●	●
Viet Nam	●	●	●	●
Zimbabwe	●	⊗	●	●

FIGURE 4.2

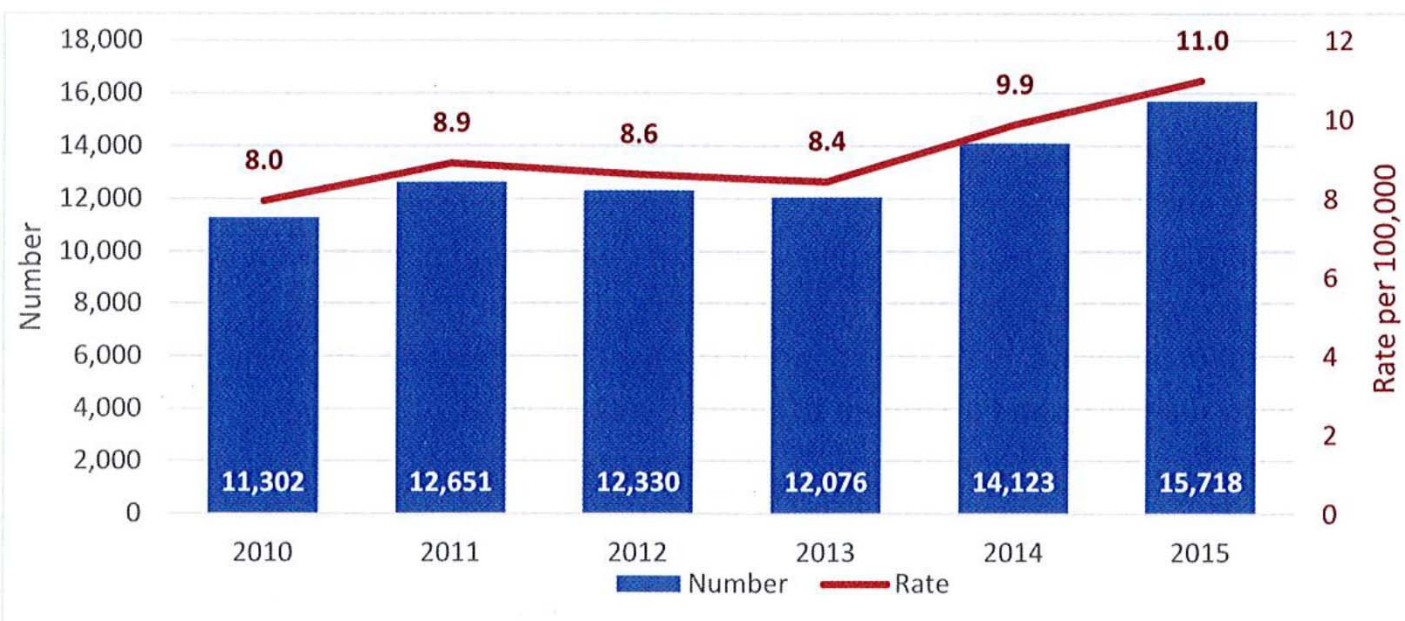
Percentage of new TB cases with MDR-TB^a



^a Figures are based on the most recent year for which data have been reported, which varies among countries. Data reported before the year 2000 are not shown.

TREND IN TOTAL NUMBER AND RATE OF ALL NEWLY NOTIFIED MDR-TB CASES, RUSSIA 2010-2015

Trend in total number and rate of all newly notified MDR-TB cases, 2010-2015



DIAGNOSTICS

- No out-of-pocket expenditures for TB tests
- Rapid molecular test (i.e. Xpert) is the initial diagnostic for all
- Second-line drug susceptibility testing (DST) is available

75% of EECA countries surveyed use Xpert MTB/RIF as the initial diagnostic test for all adults and children being investigated for TB

LPA RECOMMENDATION BY WHO

In those with **smear positive**

WHO recommends the use of LPA as a rapid diagnostic test for detection of rifampicin and/or isoniazid resistance.

The WHO recommended commercially available tests include

- GenoType MTBDR_{plus} VER 1 and 2 (Hain Lifescience, Germany),
 - The Hain version 1 and 2 assays include probes to identify *Mycobacterium tuberculosis* complex (MTBC), and detect mutations in the *rpoB* gene (associated with rifampicin resistance); in the *katG* gene and in the *inhA* promoter region (associated with isoniazid resistance). The probes used are the same for both versions of the assay.
- Nipro NTM+MDRTB detection kit 2 (Nipro, Japan).
 - The Nipro assay allows detection of MTBC and resistance to rifampicin and isoniazid. This assay also differentiates *M. avium*, *M. intracellulare* and *M. kansasii* from MTBC and from other non-tuberculous mycobacteria.



SL-LPA AND 9 MONTHS MDR-TB REGIMEN

Detect Fluoroquinolone resistance and aminoglycoside resistance

- (instead of phenotypic test)

The SL-LPA produces results in just **24-48 hours**, a vast improvement over the 3 months or longer currently required.

It allows **quick triage** of confirmed rifampicin-resistant or MDR-TB patients into either the shorter MDR-TB regimen or the conventional longer regimen or **XDR regimen**.

Excluding second-line drug resistance a critical prerequisite for identifying patients who can be placed on the shorter MDR-TB regimen.

Detection of any second-line resistance by the SL-LPA means that MDR-TB patients should not be enrolled on the shorter regimen as this could jeopardise their treatment outcome and fuel the development of XDR-TB.

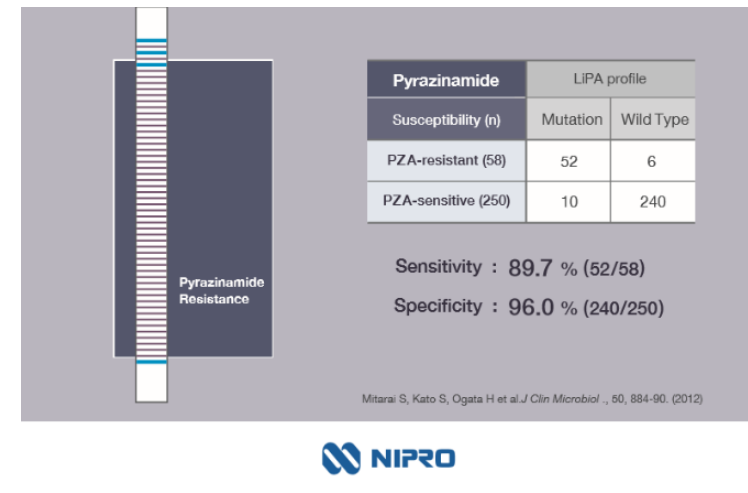
Patients detected with XDR-TB by the SL-LPA should also not be enrolled on the shorter regimen but require carefully designed individual regimens to optimise their chances of success.

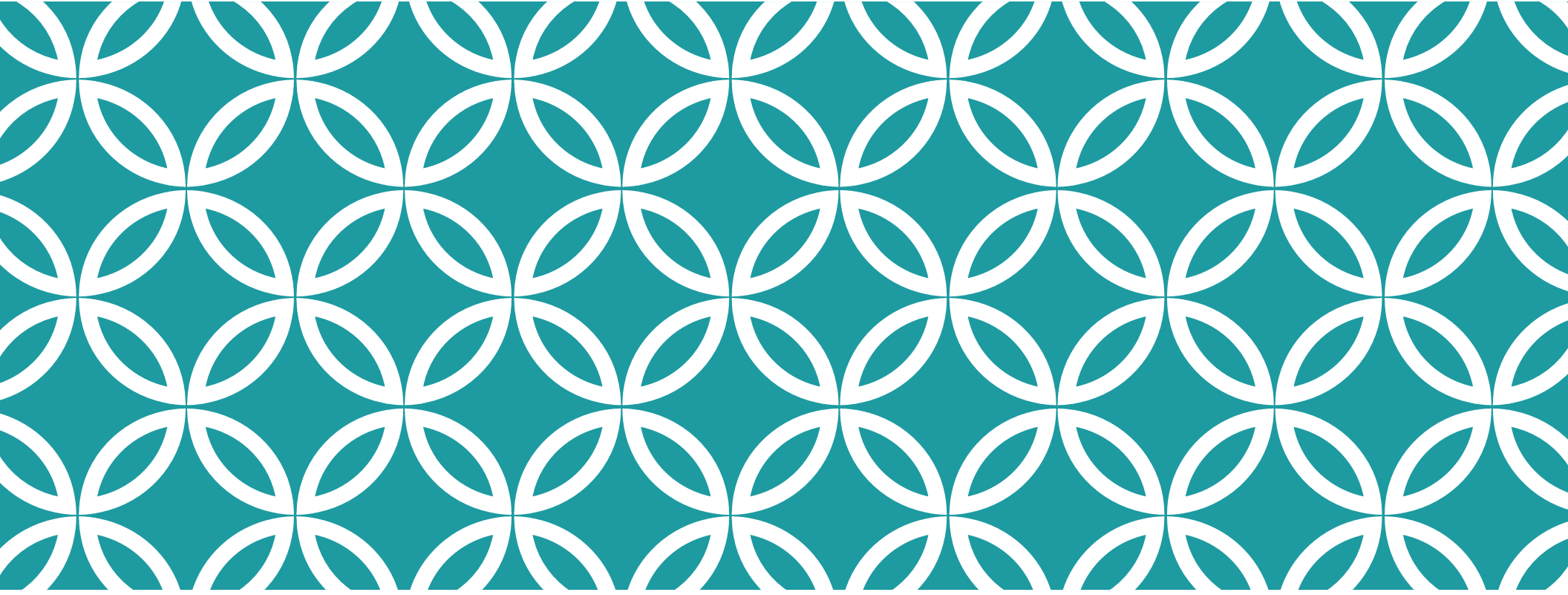
PZA RESISTANCE AND ETHIONAMIDE RESISTANCE IN 9 MONTH MDR-TB

PZA resistance has higher risk of unfavorable outcomes with 9 months short course regimens (Aung, 2014).

Knowing PZA resistance status might also help triage the patient selection and regimen in MDR-TB

LPA for PZA are available as commercial product and Accessible in Thailand/Japan (Rienthong, IJTLD, 2015)

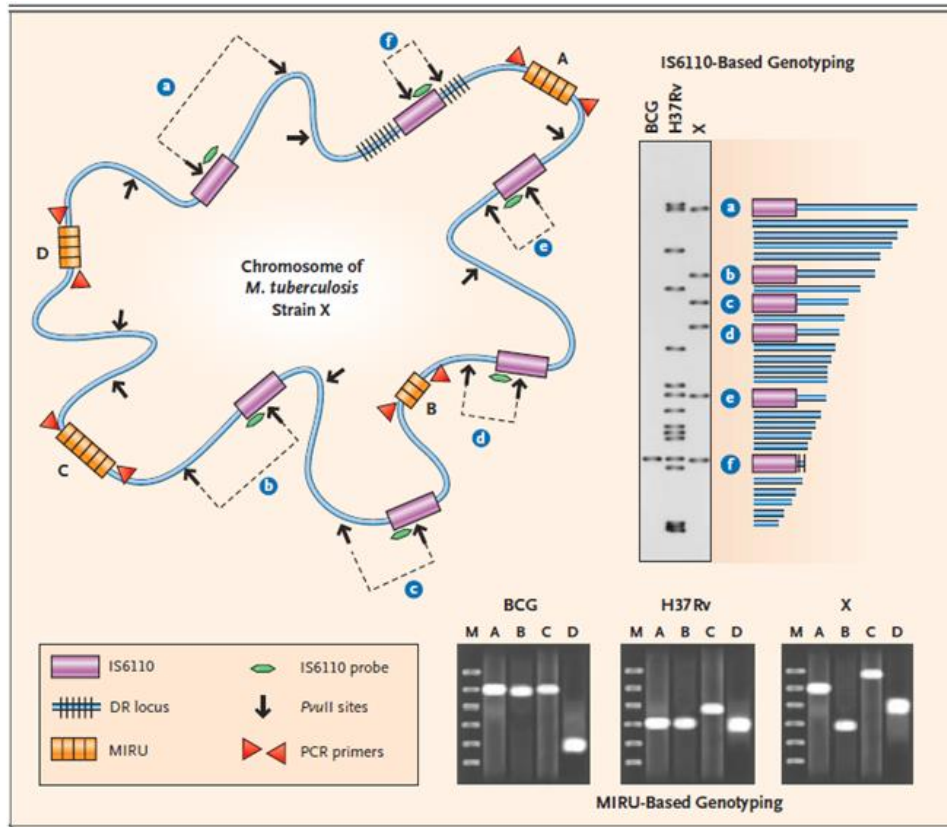




**NEW TECHNOLOGY IN MOLECULAR
INVESTIGATION OF TB OUTBREAKS**



Molecular markers for conventional genotyping of *M. tuberculosis*



Analog information

- IS6110 RFLP

Digital information

- DR locus: PCR-based spoligotyping
- MIRU: PCR based

BBC NEWS 24 MARCH 2017



British scientists claim major advance in TB treatment

A team of British scientists have made a major breakthrough in the treatment and diagnosis of tuberculosis.

Researchers in Birmingham and Oxford have been able to use genome sequencing to isolate different strains of the disease, which means patients who might have waited months to get the right drugs can now be diagnosed in little more than a week.

Public Health England said it is the first time the technique has been used on this scale anywhere in the world.

🕒 24 Mar 2017 | Birmingham & Black Country

MORE ON: Oxford

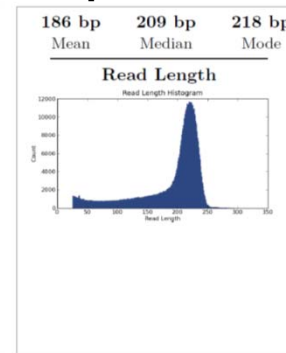
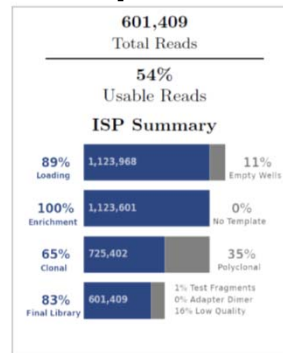
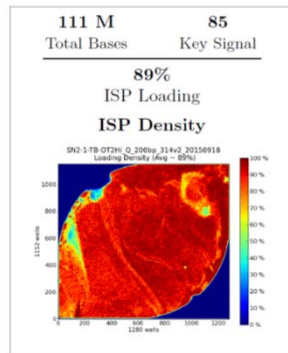
In 2016 : Whole genome sequencing of MTB

- Ion PGM sequencer (Life Technologies) has already introduced into DMSc in September 2016



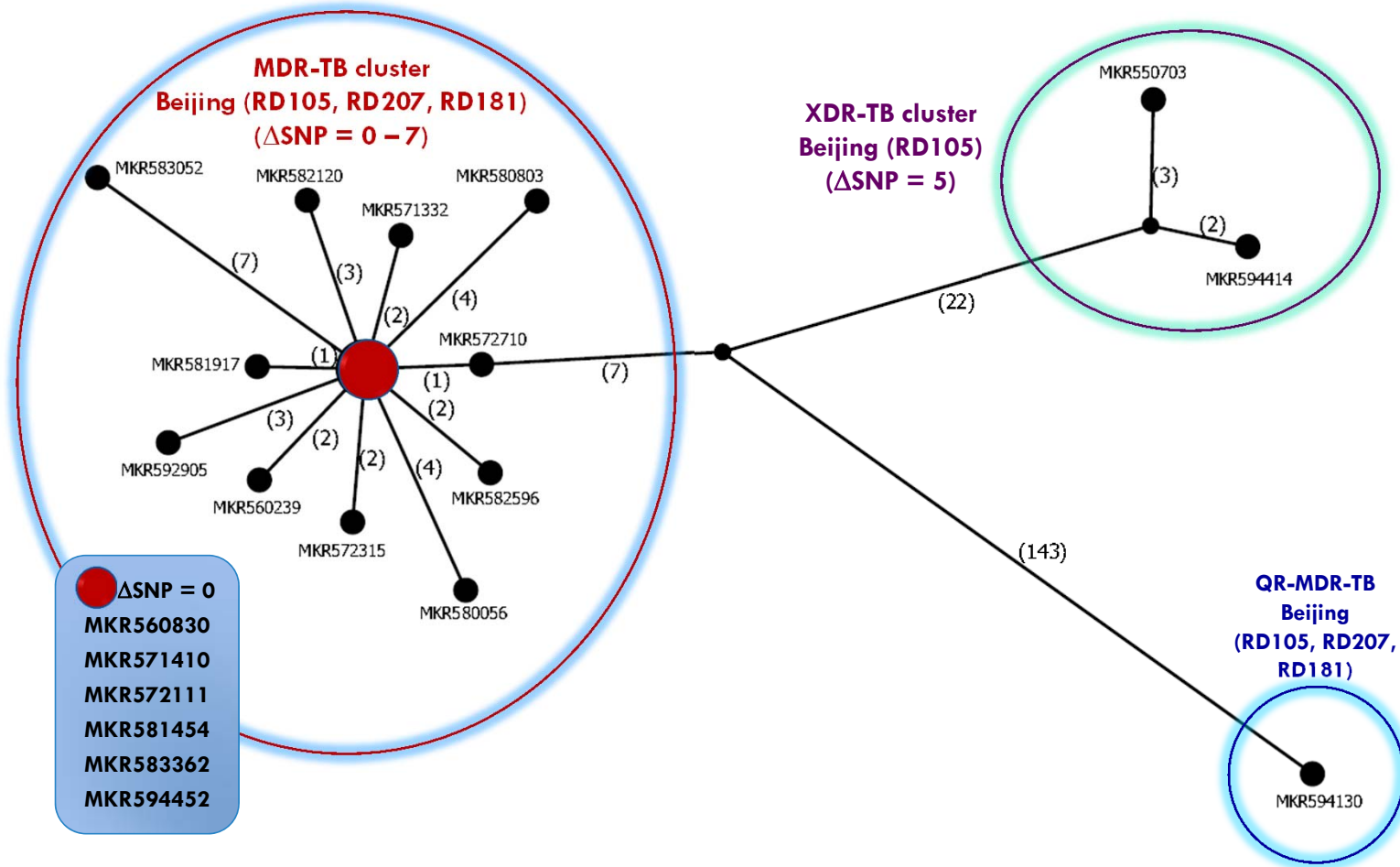
⇒ Next-generation sequencing has safely finished under the assistance of Japanese researchers.

Run Summary – 16samples in 314 chip



- Establishment of whole genome sequencing of MTB in Ion PGM.

Median-Joining Network of Beijing from patients at a district hospital in Thailand



DRUG RESISTANCE STATUS OF BEIJING STRAIN IN PATIENTS FROM CLUSTER AT MAKARAK DISTRICT

Anti-TB drugs	MDR-TB cluster	XDR-TB cluster	QR-MDR-TB
RIF	Resistance (rpoB S450L)	Resistance (rpoB S450L)	Resistance (rpoB S450L)
INH	Resistance (katG S315T: High level)	Resistance (katG S315T: High level)	Resistance (katG S315T: High level)
EMB	Resistance (embB G406D)	Resistance (embB M306I)	Resistance (embB D1024N)
STM	Resistance (rpsL K43R)	Resistance (rrs A1401G)	Resistance (rpsL K43R)
PZA	?? (pncA I31T)	Resistance (pncA I90S)	Resistance (pncA T61P)
FQs	Susceptible	Resistance (gyrA D94G)	Resistance (gyrA D94G)
AMK / KAN	Susceptible	Resistance (rrs A1401G)	Susceptible
PAS	Susceptible	Resistance (foIC E40G)	Susceptible
ETH, LZD, CFZ	Susceptible	Susceptible	Susceptible

SUMMARY

- 100% Drug susceptibility tests is mandatory for control of drug resistance tuberculosis
- Molecular based drug susceptibility tests are more practical to be implemented widely
 - RPOCT test
 - LPA
 - Other new coming tests
- Whole genome sequencing is becoming standard molecular investigating tools for MTB outbreaks in UK
- WGS is research tools that accessible to many countries