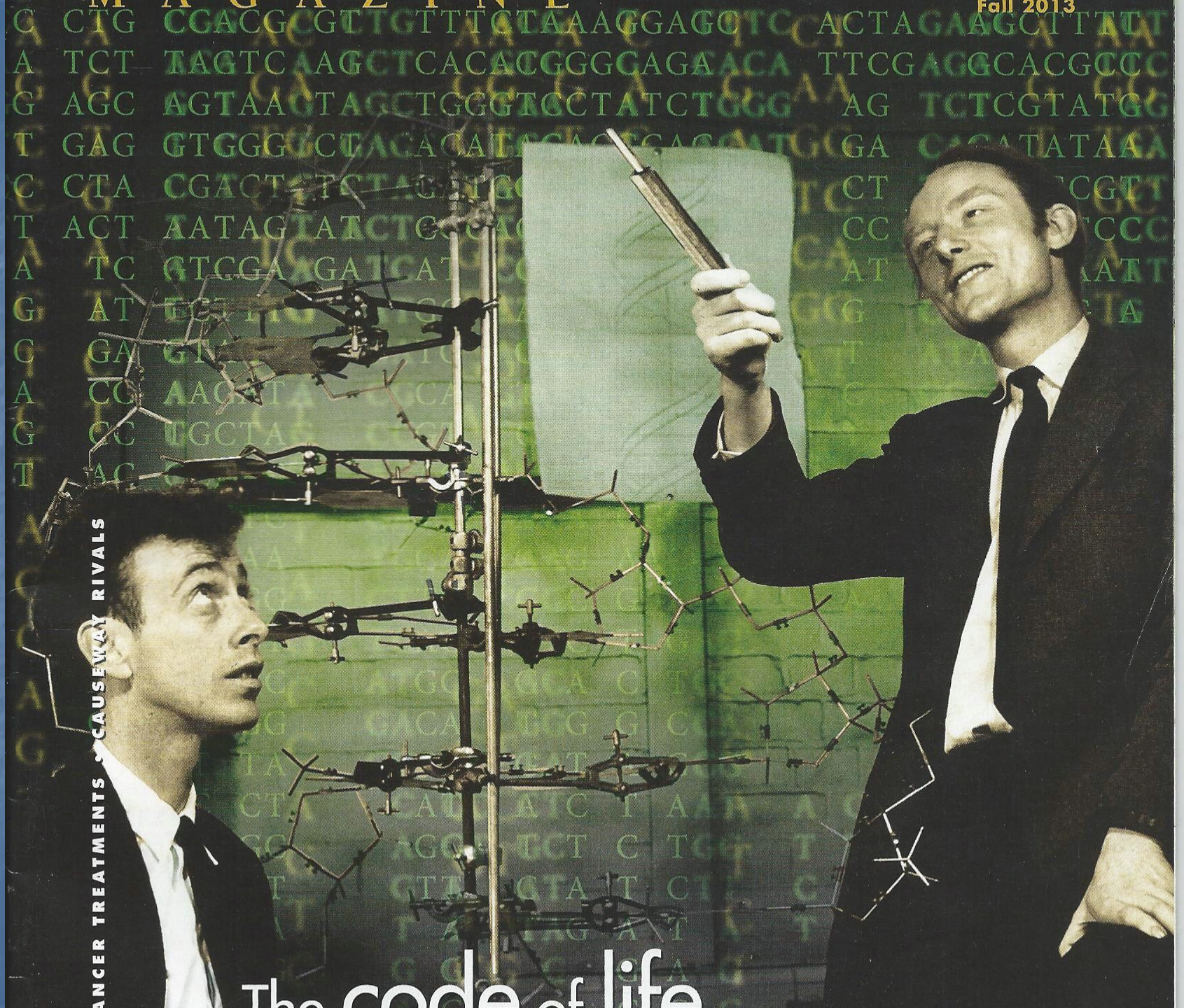
MAGAZINE
Fall 2013



CANCER TREATMENTS ✦ CAUSEWAY RIVALS

The code of life

YKHC medical staff GRAND ROUNDS:

Late to the Party:
rapid (active) TB testing in the YK Delta

Ron Bowerman. MD, MPH/ January 14, 2014

LECTURE OBJECTIVES:

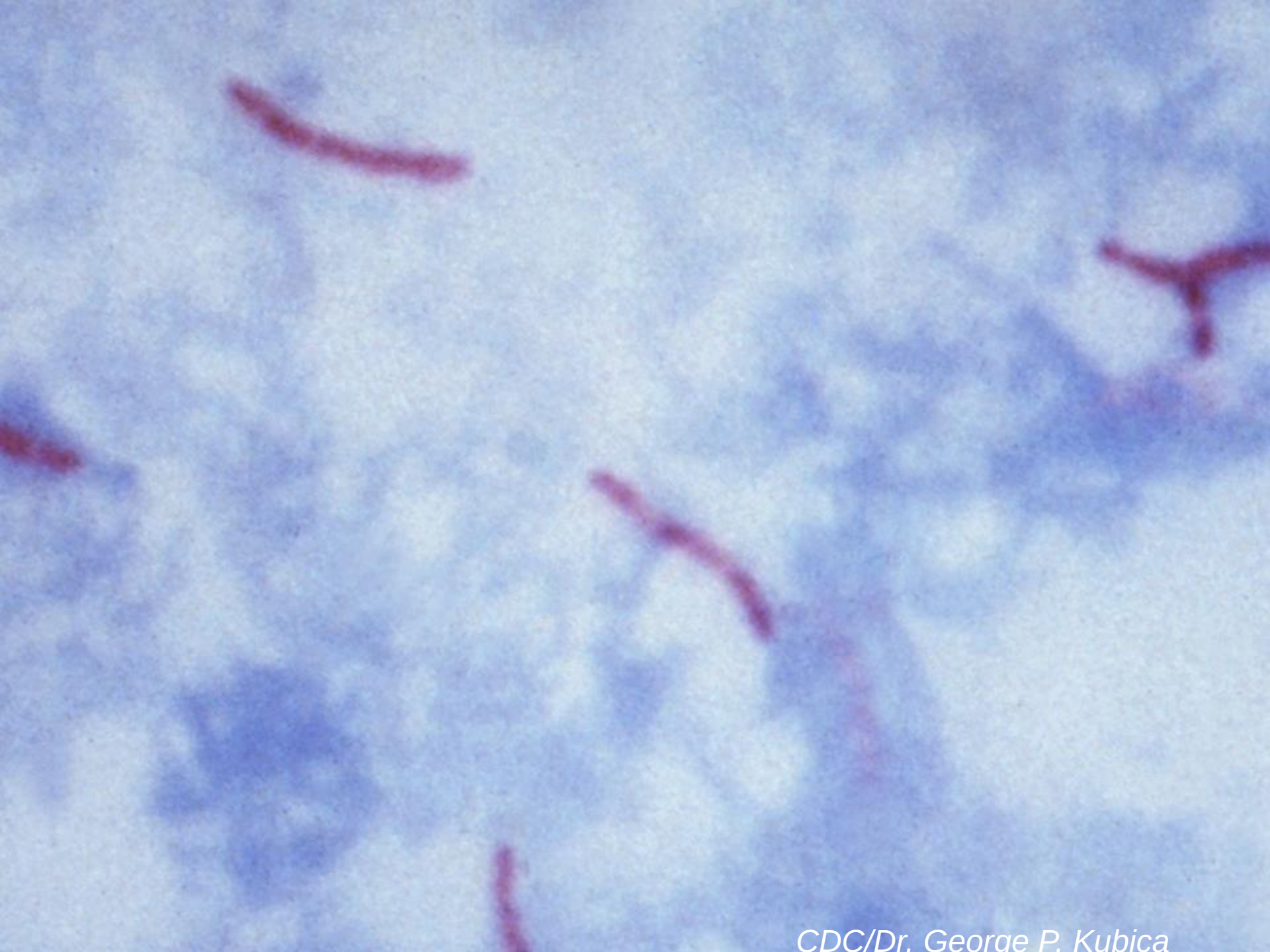
- 1. Explain what Nobel Prize-winning technology is behind this test, how it works and why it is so accurate.
- 2. Understand the current TB diagnostic limitations, especially in Bush Alaska, and how Xpert MTB/RIF will transform them.
- 3. Make a case for improving Human Rights in Bush Alaska by implementing this test.
- 4. Understand why Alaska is so late in having access to this test while half the world has been using it for the past two years and especially, in which country?

This lecture is not about QuantiFERON

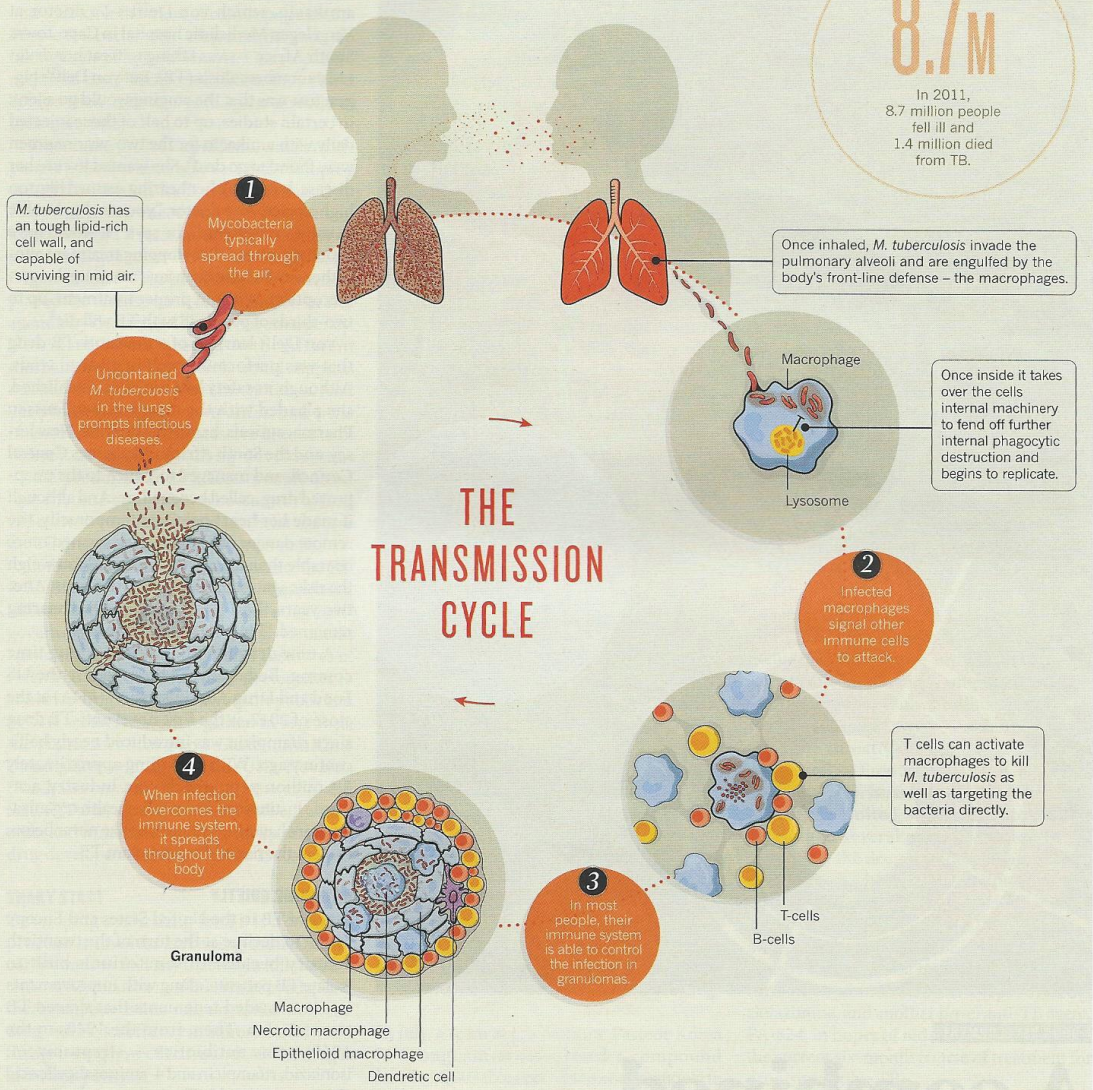

Tuberculosis
a quick review

Mycobacterium tuberculosis

- Obligate aerobe
- Facultative intracellular pathogen
- Infects macrophages
- Slow growing
- Hydrophobic→unable to gram stain
- Acid-fast bacilli



CDC/Dr. George P. Kubica



1

Transmission

People with active disease develop symptoms such as a cough, which propels bacteria into the air where it can be inhaled by others. Tuberculosis must be diagnosed and treated as soon as possible to render the person non-infectious and prevent the spread of disease (see 'Control issues', page S16).

2

Immune response

The BCG vaccine offers little protection to adolescents and young adults from the form of the disease that causes most deaths. Any effective vaccine will need to harness T cells, but scientists are still looking for correlates of protection (see 'An age-old problem', page S8).

3

Latency

M. tuberculosis can be contained within granulomas for years. It is thought that latency may encompass a spectrum of states, from people who have completely controlled the disease, to those with undetected, subclinical disease (see 'Latency: A sleeping giant', page S14).

4

Activation

If the immune system weakens as a person ages or contracts HIV infection, for example, bacterial replication can overcome the immune system and granuloma breaks down, releasing *M. tuberculosis* into the lungs and advancing disease (see 'A combined effort', page S4).

WORLD TUBERCULOSIS REPORT 2012. LIENHARDT, C. ET AL. NATURE REVIEWS MICROBIOLOGY 10, 407-416 (2012)

Robert Koch

German physician/microbiologist

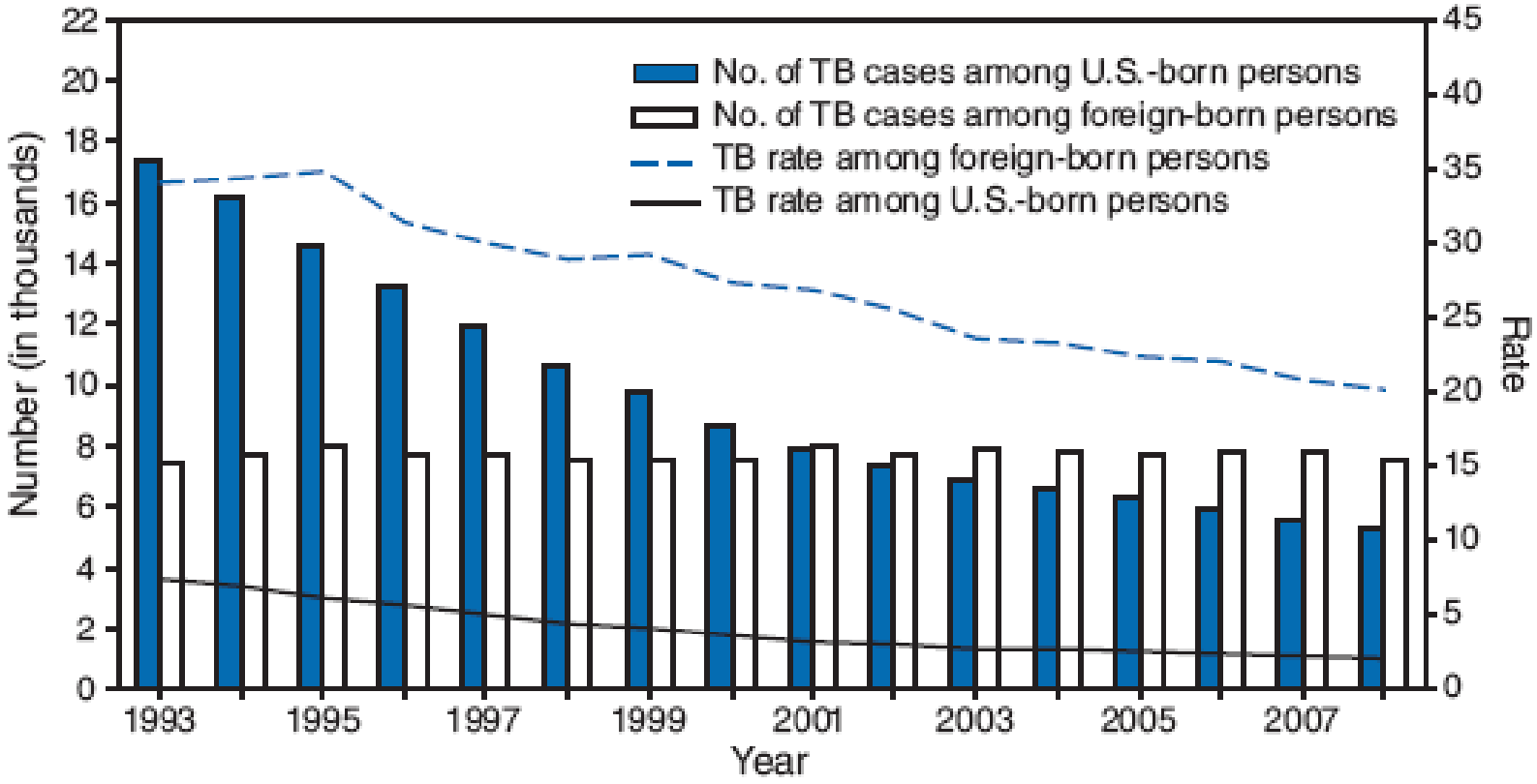
1843 (May 24) -1910

1882 discovered tubercle bacilli

1896 discovered tuberculin

1910 received Nobel Prize in Medicine

FIGURE 2. Number and rate* of tuberculosis (TB) cases among U.S.- and foreign-born persons, by year reported — United States, 1993–2008†



SOURCE: National TB Surveillance System.

* Per 100,000 population.

† Data are updated as of February 18, 2009. Data for 2008 are provisional.

"The past 10 years have seen the most rapid growth in new diagnostics for Mtb in over a century."

Nyendak MR, Lewinsohn DA, Lewinsohn DM 2009. New diagnostic methods for tuberculosis. *Current Opinion in Infectious Diseases* 22 (2): 174-182.

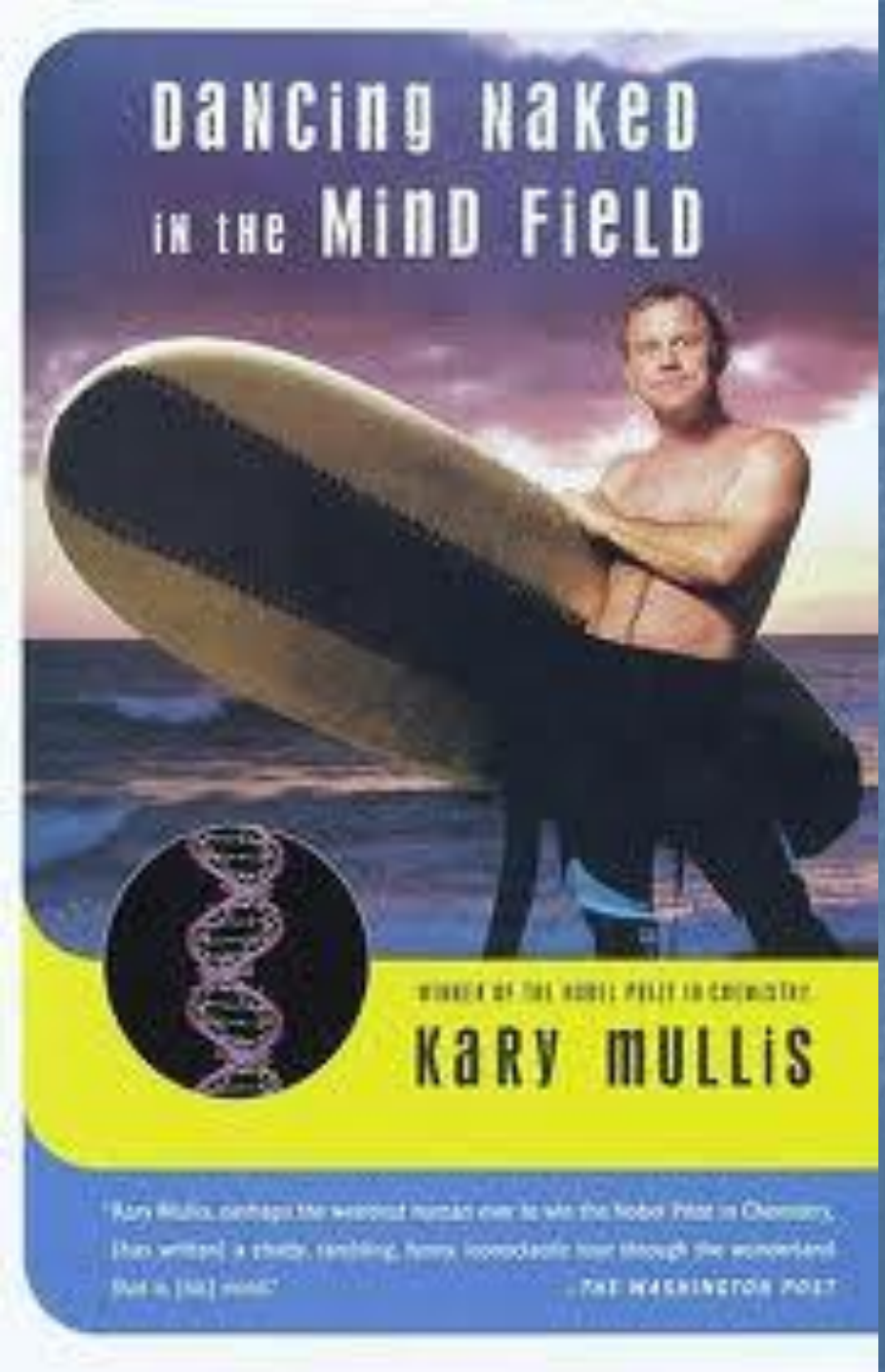
Xpert MTB/RIF test

- -separation of TB and Rifampin resistant gene sequences
- -amplification by PCR
- -ID by molecular beacons
- -very QUICK and PORTABLE

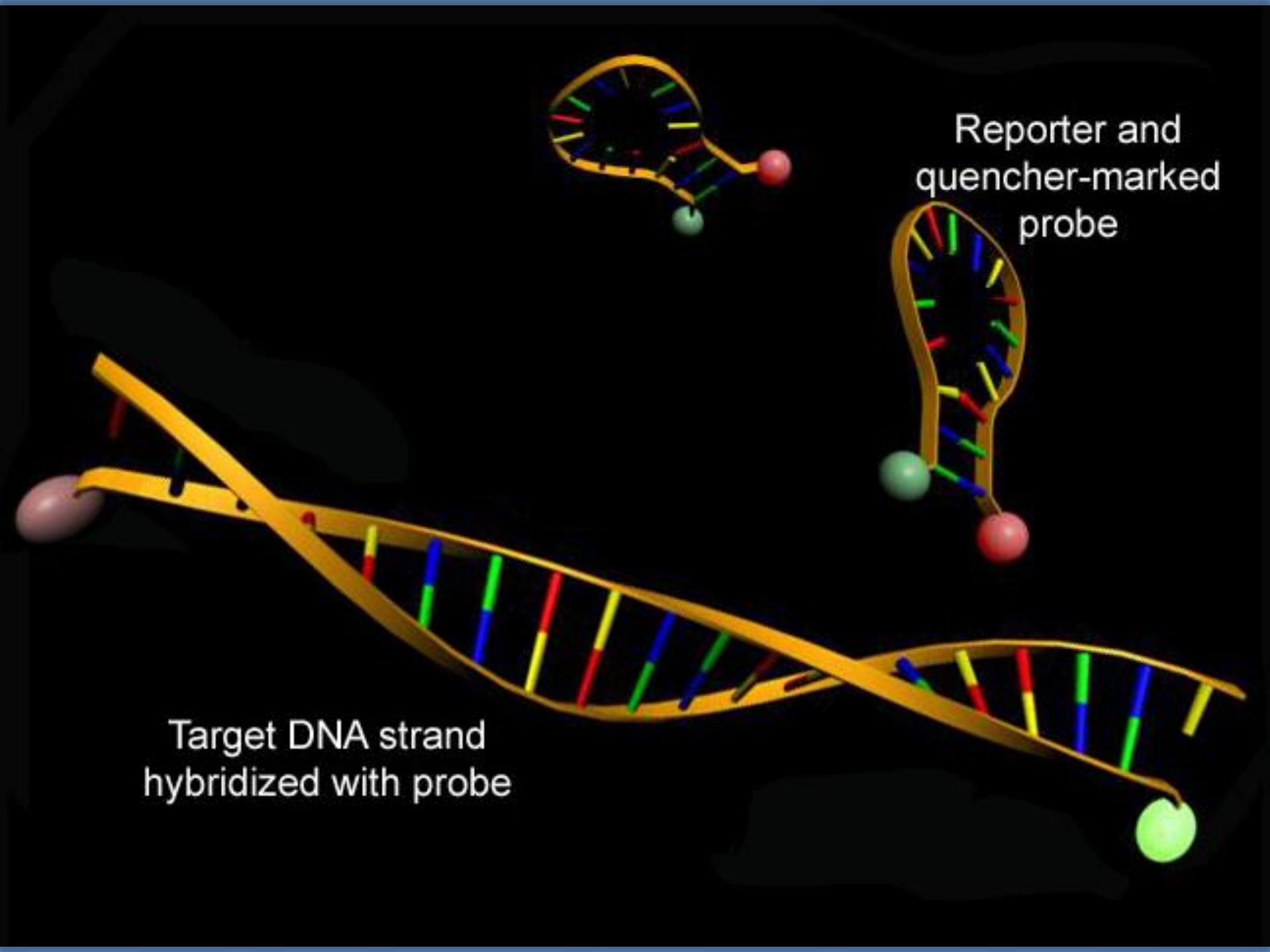
Dr. Kary Mullis

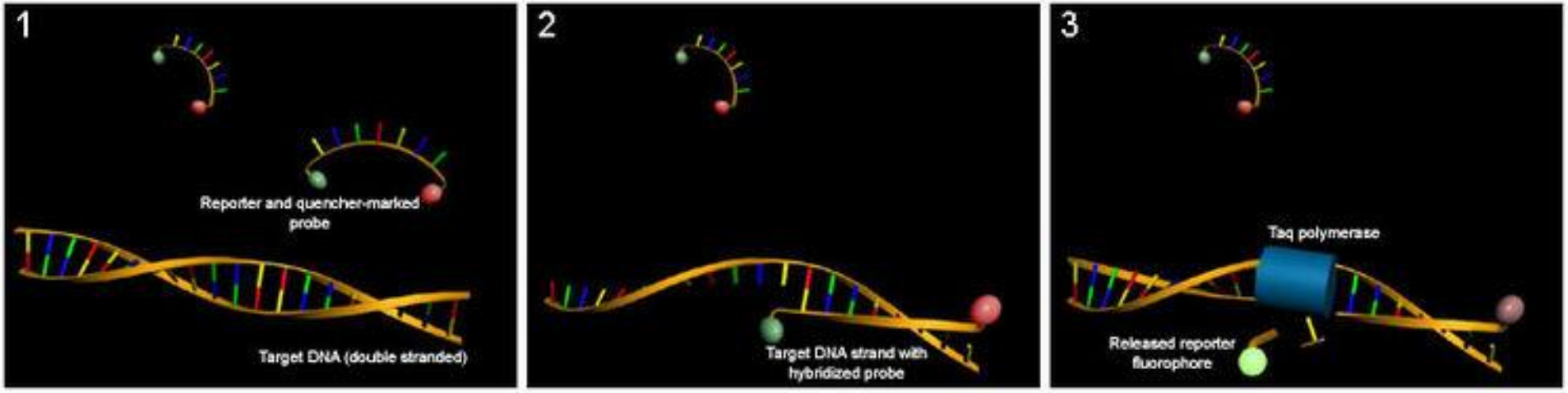
Nobel Prize
Chemistry 1993

Inventor of PCR process



Kary Mullis, PhD receiving Nobel prize in Stockholm


Reporter and
quencher-marked
probe
Target DNA strand
hybridized with probe


1
Reporter and quencher-marked probe
Target DNA (double stranded)
2
Target DNA strand with hybridized probe
3
Taq polymerase
Released reporter fluorophore

Why a new Rapid TB diagnostic test?

Why a new Rapid TB diagnostic test?

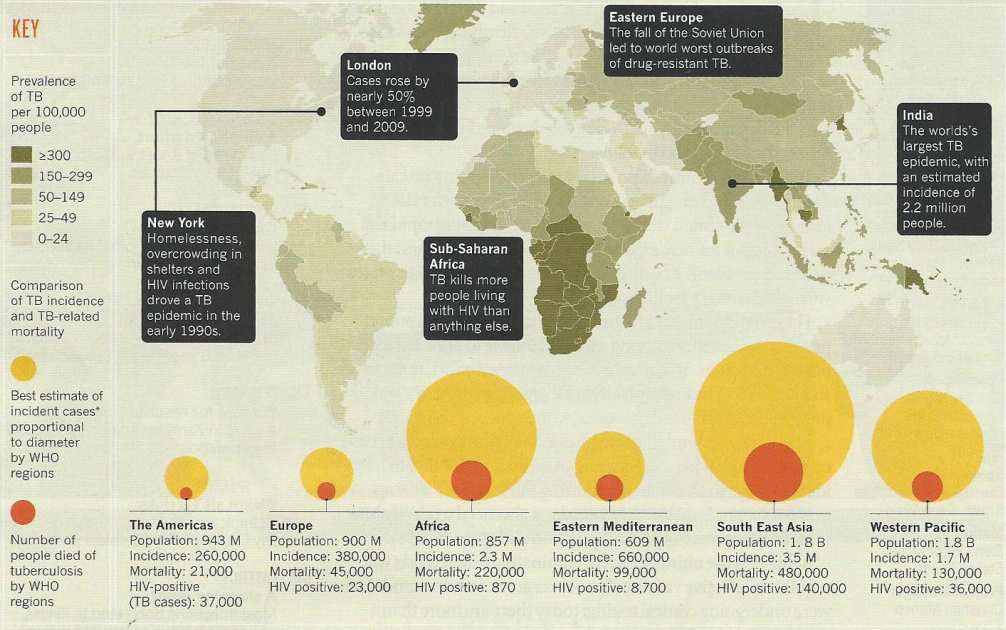
- The worldwide battle against TB is going poorly

A MORTAL FOE

Tuberculosis is one of the world's most lethal, infectious diseases. Further progress in consigning it to the past is a massive challenge. By **Tom Paulson.**

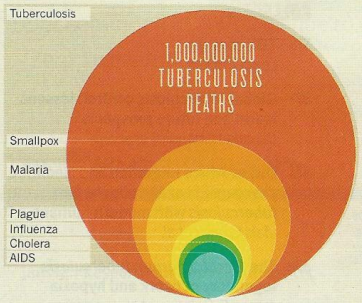
GLOBAL BURDEN OF TUBERCULOSIS

In 2011, nearly 9 million people fell ill from TB and 1.4 million died, mostly in poor countries, with 60% of cases in Asia and 24% in Africa.



THE BIGGEST KILLER

Tuberculosis has killed more than any other infectious disease in history. Over a billion lives in the past two hundred years.

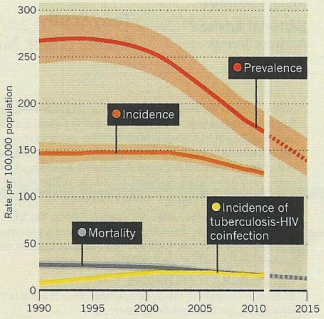


THE 100 YEARS BATTLE

Rising living standards in industrialized nations, interupted by two World wars, and new antibiotics had tuberculosis in decline.

SLOW PROGRESS

Over the past fifteen years, an invigorated anti-TB effort has begun to reduce the global burden of disease worsened by HIV.



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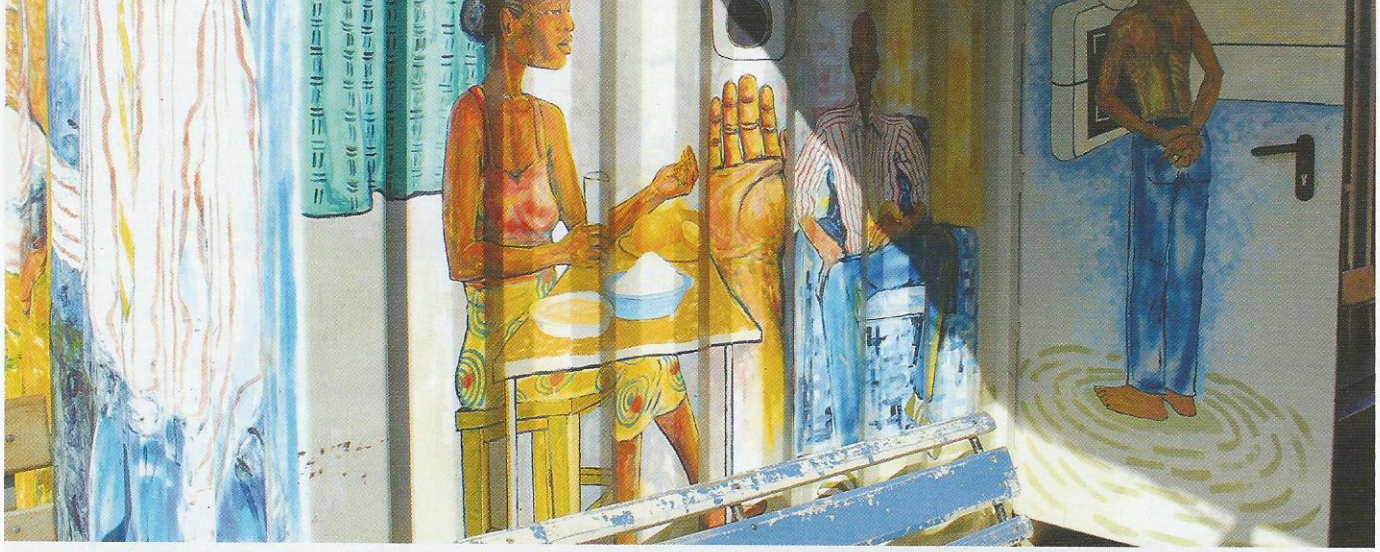
- The current "wait" in TB dx/tx prevents early intervention



The health clinic in Kanyama, a slum district of Lusaka, Zambia, is trying out a new device to test for tuberculosis.

DIAGNOSIS

Waiting for results

There are several new tests for tuberculosis in the pipeline, but they must be shown to be effective in areas with limited resources and a heavy burden of HIV.

BY CATHERINE DE LANGE

Tobias Hamooya says he is feeling a bit better these days, but even to the casual observer it is clear that he is still not doing well. Sitting on the porch outside the Macha Mission Hospital in rural Zambia, his slow, staggered speech is punctuated by a violent cough, and just talking seems to leave him drained.

A few weeks ago Hamooya's cough got so bad he left his wife to look after their newborn baby and six other children and travelled the 150 kilometres to get here. Did he have any idea what was wrong with him? His answer needs no translation: "TB". Since arriving at the clinic they also tested him for HIV, and the results came back positive for that too. Once his tuberculosis (TB) medication kicks in, he will begin it. You may go all your life and never have a problem. Now with HIV, which destroys the immune system, it becomes a big issue."

The highest proportion of new TB cases is in sub-Saharan Africa: more than 260 people per 100,000 in 2011. By comparison, in the same year France saw 4 cases per 100,000. And the region is in the grips of an HIV epidemic; TB kills more people living with HIV than anything else, and detection and treatment of TB is vastly complicated by HIV co-infection. Where once treatment programmes operated independently, many countries like Zambia now try to test and treat the two together. In 2004, just 3% of TB patients in the World Health Organization (WHO)'s African Region were tested for HIV; in 2011, it was 69%. Hamooya was one of the lucky ones: detecting TB is extremely difficult in patients who also have an HIV infection.

public-health goal, and these efforts are beginning to bear fruit. Over the next few months, a new test called Xpert MTB/RIF that detects TB DNA will enter clinics in several high-burden countries in southern Africa; other tests are close behind. The question is, in the uncompromising settings that are home to the greatest burden of disease, will these new tests fulfil their potential?

NEED FOR SPEED

A fast TB diagnosis is important. The sooner a patient is diagnosed, the sooner they can start treatment to mitigate the debilitating symptoms of TB and limit the potential for transmission. But there is an added imperative for HIV-positive patients. "Starting someone on ARVs who has [untreated] TB means that, as their immune system

Why a new Rapid TB diagnostic test?

- The worldwide battle against TB going poorly
- To end TB by 2050 we need 16% annual decline
- The current decline trend is 2%
- HIV and MDR/XDR TB compounds this effort
- 1/3 of new TB cases are being missed annually
- The wait in TB dx/tx prevents early intervention

- The Xpert MTB/RIF test is highly effective, simple, FAST, and addresses drug resistance

Video: new TB device

Xpert® MTB/RIF

Two-hour detection of MTB and resistance to rifampicin.

Go from test and wait
*to **test and treat**.*

CE IVD In vitro diagnostic medical device

defining *on-demand* molecular diagnostics.

Cepheid.
Bring answers to life.

History of Xpert MTB/RIF Assay

- GeneXpert diagnostic system developed by Cepheid (Sunnyvale, CA)
- First deployed by USPS for rapid detection of ANTHRAX in mail sorting offices

Ulrich MP, Christenson DR, Coyne SR, et al. J. Appl Microbiol 2006; 100: 1011-16

Description: Xpert MTB/RIF Assay

"It is a self-contained, fully integrated, automatic platform that can be used with minimal technical skills. The cartridge-based system incorporates microfluidics technology and fully automated nucleic acid analysis to purify, concentrate, detect, and identify targeted nucleic acid sequences from unprocessed clinical samples."

Lawn SD, Nicol MP. Future Microbiol 2011; 6: 167-82

Microfluidics Technology

is a multidisciplinary field intersecting engineering, physics, chemistry, nanotechnology and biotechnology, with practical applications to the design of systems in which small volumes of fluids will be handled. Microfluidics emerged in the beginning of the 1980s and is used in the development of inkjet printheads, DNA chips, lab-on-a-chip technology, micro-propulsion, and micro-thermal technologies.

From Wikipedia, the free encyclopedia

Video: gas

GeneXpert test platforms by Cepheid


Photo courtesy of Cepheid

Xpert MTB/RIF test locations in PNG

Xpert MTB/RIF test

-cartridge-based, automatic diagnostic test

-detects DNA sequences specific to

-Mycobacterium tuberculosis

-Rifampin resistance mutations

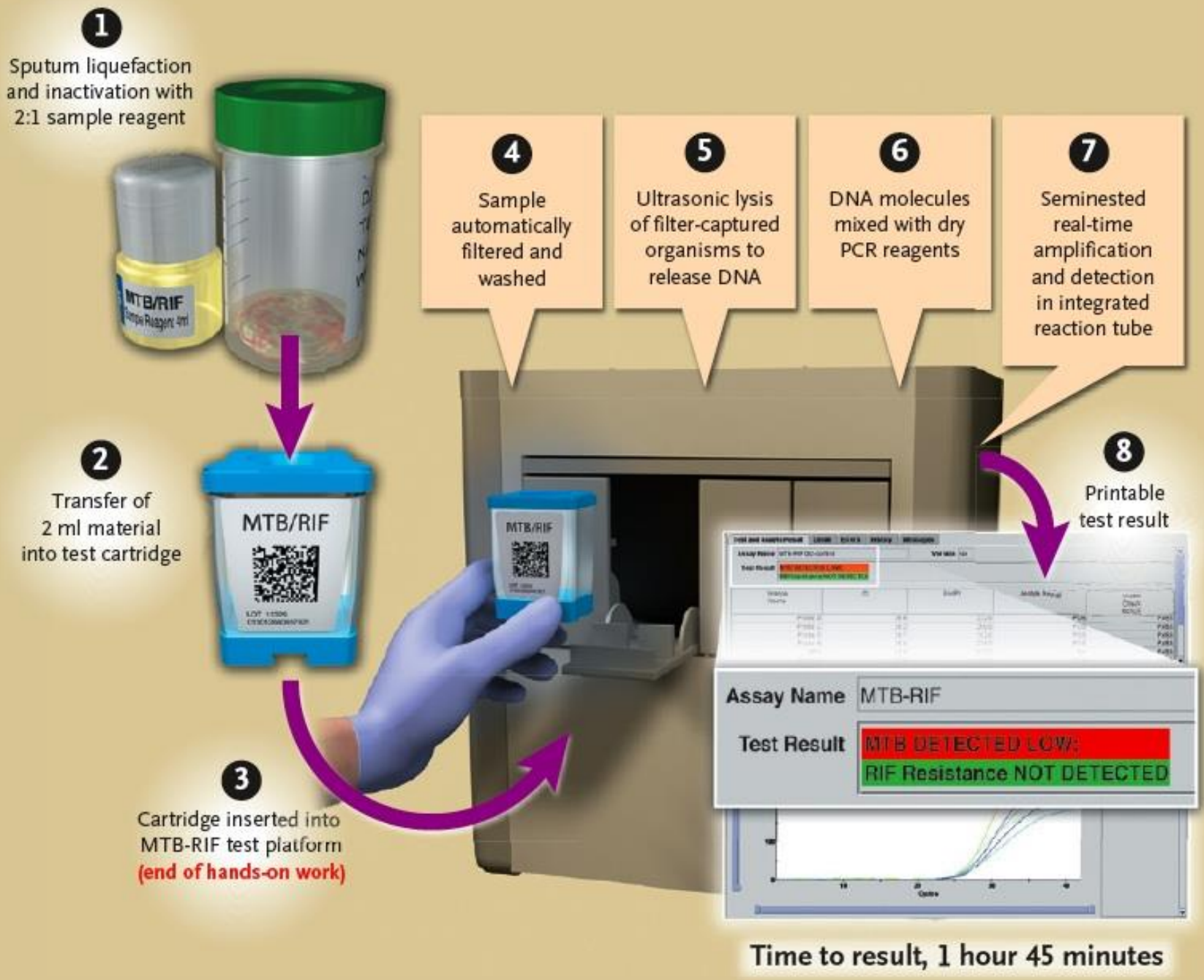
2010 endorsed by WHO for use in TB endemic areas

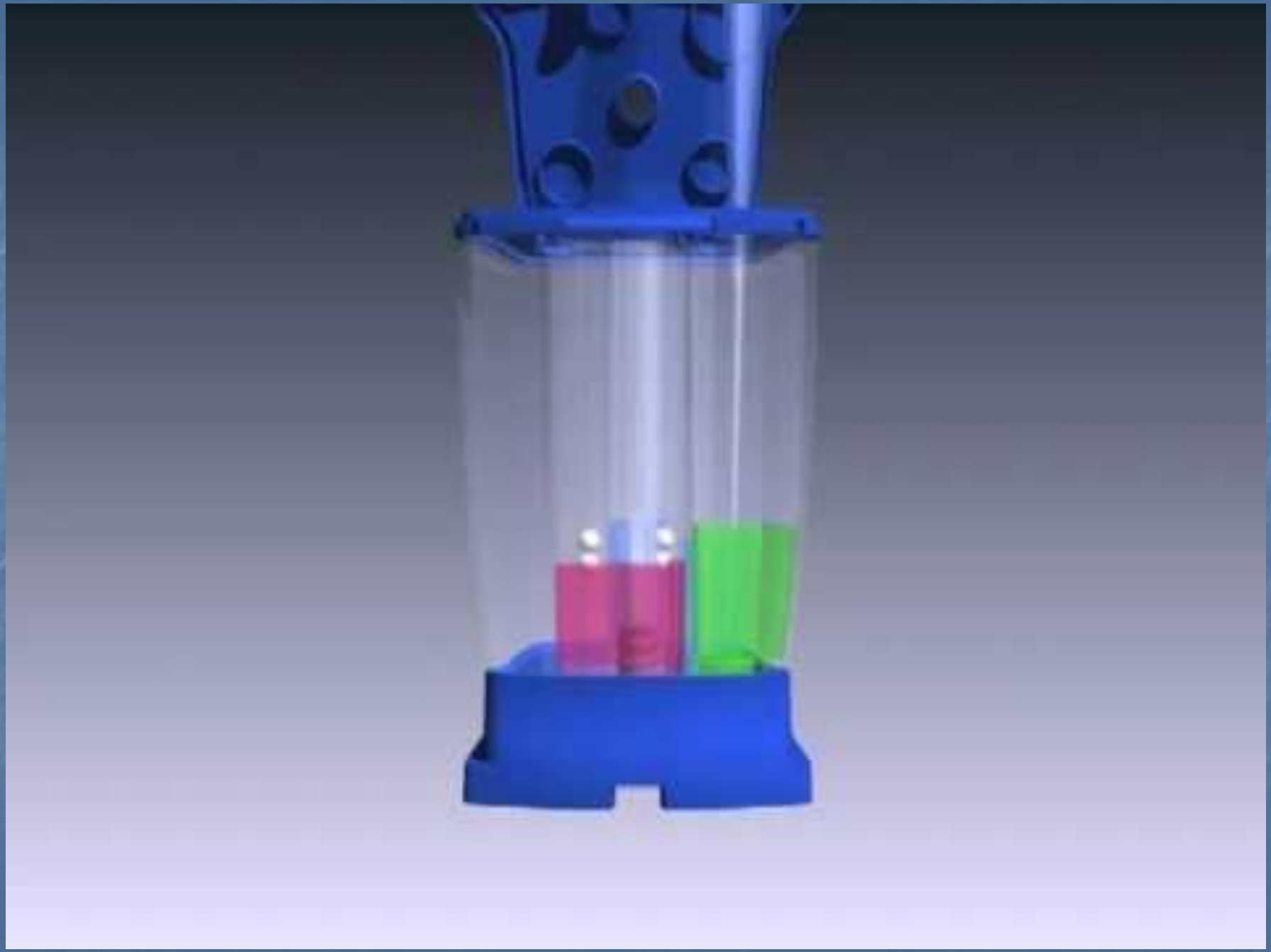
2013 FDA-approved & CLIA-endorsed in the US


GeneXpert
Xpert® MTB/RIF

Xpert MTB/RIF test

- Results from unprocessed sputum sample
- Very low level bio-hazard
- Little technical training (CLIA mod complx)
- Sputum→result as soon as 90 minutes


1
Sputum liquefaction and inactivation with 2:1 sample reagent
MTB/RIF
2
Transfer of 2 ml material into test cartridge
MTB/RIF
3
Cartridge inserted into MTB-RIF test platform
(end of hands-on work)
4
Sample automatically filtered and washed
5
Ultrasonic lysis of filter-captured organisms to release DNA
6
DNA molecules mixed with dry PCR reagents
7
Seminested real-time amplification and detection in integrated reaction tube
8
Printable test result
Assay Name
MTB-RIF
Test Result
MTB DETECTED LOW
RIF Resistance NOT DETECTED
Time to result, 1 hour 45 minutes



Video: cartridge

GeneXpert Cartridge.lnk

Xpert MTB/RIF Test Endorsed

- WHO-endorsed 12/2010 as equivalent to AFB smear test for diagnosis of active TB (currently in use in >87 countries)
- FDA-approved 2013
- CLIA-endorsed 2013 for general lab use

Xpert test Accuracy for Pumonary TB

for single sputum samples

- Sensitivity of smear (+) TB pts : 98.2%
- Sensitivity of smear (-) TB pts: 72.5%
- Specificity (i.e. true neg result): 99.2%

Boehme CC, Nabeta P, Hillemann D. N Engl J Med 2010; 363: 1005-15
(n=1730 suspected TB patients from S.Africa, Peru, Azerbaijan, and India)

and 98·7%, respectively. Data published after this date have broadly similar findings. A modified G4 version of the cartridge was launched in December, 2011, and independent data on the diagnostic accuracy of this version are needed.

39–81) to 5 days (2–8). Rates of untreated smear-negative culture-positive tuberculosis decreased from 39·3% without the Xpert MTB/RIF assay to 14·7% with the assay. Assay performance for detection of rifampicin resistance was also excellent, with a median time to

	Country	TB reference standard diagnoses (samples)	TB not diagnosed (samples)	Main sample types testing positive for TB (samples)	Reference standard for TB diagnosis	Xpert MTB/RIF assay sensitivity for TB, % (95% CI)	Xpert MTB/RIF assay specificity, % (95% CI)
Testing of various extrapulmonary samples for TB							
Armand et al, 2011[31]	France	32	NA	Lymph nodes (16), pleural fluid (7), bone (5)	Culture (solid and liquid media)	53·1% (34·7–70·9)	NA
Causse et al, 2011[32]	Spain	41	299	Tissue biopsy samples (18), CSF (6), gastric aspirates (8), pleural fluid (4), purulent exudates (5)	Culture (solid and liquid media)	95·1% (83·5–99·4)	100% (98·8–100)
Friedrich et al, 2011[33]	South Africa	20	5	Pleural fluid (25)	Culture (liquid media)	25·0% (8·7–49·1)	100% (47·8–100)
Hillemann et al, 2011[34]	Germany	45	476	Tissue (30), gastric aspirate (8), urine (5)	Culture (solid and liquid media)	77·3% (60·5–87·1)	98·2% (96·0–98·9)
Ligthelm et al, 2011[35]	South Africa	30	18	Fine needle aspiration lymph node biopsy	Composite standard: positive cytology + acid-fast bacilli and/or culture of TB	96·6% (86·6–100)	88·9% (69·6–100)
Moure et al, 2012[36]	Spain	108	41	All smear-negative, pleural fluid (26), lymph nodes (34), abscess aspirates (17), tissues (12)	Culture (solid and liquid media)	58·3% (48·5–67·8)	100% (91·4–100)
Vadwai et al, 2011[37]	India	283	250	Tissue biopsy samples (105), pus (98), body fluids (24)	Composite of smear, culture, clinical, radiology, and histology	80·6% (75·5–85·0)	99·6% (97·8–100)
Zeka et al, 2011[38]	Turkey	48	128	Pleural fluid, lymph node biopsy, CSF, urine, skin biopsy samples, pericardial fluid	Culture (solid and liquid) or suggestive clinical features, radiology or histology	54·2% (40·3–67·4)	100% (97·2–100)
Tortoli et al, 2012[39]	Italy	268	1206	Tissues biopsies or fine needle aspirates (94), pleural fluid (18), gastric aspirates (61), pus (55), CSF (14), urine (16), peritoneal, synovial, or pericardial fluid (10)	Culture (solid and liquid) or suggestive radiology or histology with documented positive response to TB treatment	81·3% (76·2–85·8)	99·8% (99·4–100)
Testing of urine samples from patients infected with HIV with culture-positive pulmonary TB							
Lawn et al, 2012[40]	South Africa	84 outpatients screened before antiretroviral therapy	NA	2·0 mL of urine	Sputum liquid culture	Overall: 19·0% (11·3–29·1); CD4 <50: 44·4%; CD4 50–150: 25·0%; CD4 >150: 2·7%	NA
Peter et al, 2012[41]	South Africa	113 inpatients	62	1·0–10·0 mL of urine (+/– centrifugation)	Liquid culture of sputum or extrapulmonary sample	Overall: 47·8% (38·8–56·9); CD4 <200: 53·8%; CD4 >200: 30·8%	98% (95–100)

Only studies with at least 20 reference standard diagnoses of extrapulmonary tuberculosis were included. TB=tuberculosis. MTB=*Mycobacterium tuberculosis*. RIF=rifampicin. NA=not applicable.

Table 1: **Summary of studies of the diagnostic accuracy of the Xpert MTB/RIF assay for extrapulmonary tuberculosis**

into their revised diagnostic policies. Up to the end of June, 2012, 1·1 million test cartridges were procured by 67 (46%) of the 145 countries eligible to purchase them

***Panel 2:* Key strengths and weaknesses of the Xpert MTB/RIF assay**

Strengths

- Robust
- Good accuracy for tuberculosis diagnosis
- Simple to use
- Rapid (2 h) compared with existing tests
- Detects both *Mycobacterium tuberculosis* complex and rifampicin resistance
- Better sensitivity and specificity than smear microscopy
- Does not need advanced biosafety equipment
- Closed system with low risk of cross-contamination
- Could potentially be used to test a broad range of samples from extrapulmonary sites—eg, lymph node aspirates, gastric lavage, urine, and CSF (not yet endorsed by WHO; more data awaited)
- GeneXpert platform is multifunctional and could be used for other diagnostics such as HIV viral load
- Modular platform permits capacity to match demand in a given facility
- Operators do not need formal laboratory training

Weaknesses

- Expensive
- Sophisticated hardware needing calibration and maintenance and linkage to a computer and secured premises
- Operators need training in basic computer skills
- Needs continuous electrical power supply and air conditioning
- Storage of samples at room temperature restricted to 3 days
- Relatively short shelf life of reagent cartridges needing good procurement systems
- Need for cartridge storage at 2–28°C and system for disposal after use
- Although comparatively rapid, the turnaround time is a challenge for same-day diagnosis and treatment in overcrowded health facilities
- False-positive rifampicin resistance results
- Cannot differentiate between live and dead *M tuberculosis*, thus cannot be used to monitor treatment success or failure, or relapse
- Cannot differentiate between *M tuberculosis*, *M bovis*, and BCG vaccine
- Use with extrapulmonary samples is not yet fully defined

take a step-wise approach to introduction an
beginning with the establishment of an
coordination mechanism, such as an Xpert
assay technical working group or advisory t
working groups should include representatio
key stakeholders, including national tuberc
AIDS control programmes, national pub
laboratories and supranational tuberculosis
laboratories, implementing partners, and c
should be tasked with leading the strategic
implementation, and assessment process
mentation plans should consider the local epi
available diagnostic services and laborator
and first-line and second-line drug treatmer
Moreover, implementation should be in
relevant strategic plans (eg, national tuberc
AIDS control programmes and national
strategic plans). Furthermore, implementat
be closely linked to monitoring and asse
clearly defined outcome measures to inform
in procedures, policies, and plans.

South Africa has led the way with natio
mentation of the Xpert MTB/RIF assay.
African Ministry of Health has rec
replacement of smear microscopy as the initia
test for tuberculosis. This step is unlikely to
other countries in the region in the foresee
because of cost and logistical constraints.
2012, South Africa accounted for 37% of the m
53% of the cartridges procured globally.[1] In M
the National Department of Health announc
to achieve national scale-up over 2–3 years.
African National Health Laboratory Service
pilot programme, placing GeneXpert pla
25 smear microscopy centres across the co
throughputs ranging from 16 to more than 4
day.[73] Following this successful pilot, machin
being placed in all existing smear
laboratories, fully replacing smear micro
diagnosis of pulmonary tuberculosis in Sou

Test Applications in the YK Delta

- More efficient TB medical care
- Improved TB patient/Human Rights
- Improved Hospital/Community Safety
- Reduced Healthcare Costs

Current Way to Dx TB in YK Delta

Currently, the AFB test can only be done in the State Epi Lab in Anchorage despite our Lab trying to bring it back to Bethel over the last 10 years. This has been a huge inconvenience to our TB medical care in the YK Delta, not to mention human-rights issues as we keep patients unnecessarily hospitalized awaiting State Lab results to "rule-out TB."

Current Way to Dx TB in YK Delta

Also, the waste in hospitalization-special isolation cost is enormous. The Xpert MTB/RIF assay would allow us to "leap-frog" the off-site AFB problem to more nimbly and efficiently battle TB and additionally provide a built-in surveillance for multi-drug resistant TB with the RIF test component.

The Xpert MTB/RIF assay has the potential to impact medical care, patient rights, safety and costs as follows:

Test Applications in the YK Delta

- More efficient TB medical care

Like our CT scanner, the greater immediate diagnostic clarity of this rapid and very accurate TB assay will promote better care. This is how it might be used in the ER setting:

POSITIVE rapid sputum test result = Active Pulmonary TB and Start 4-drug TB meds, also,

- 1. Stable/Bethel pt→send home on 4-drugs with mask and PHN f/u
- 2. Stable/Village pt→admit to NWing-TB isolation on 4-drugs, send home when AFB x3 Neg (as before)
- 3. Unstable/Bethel pt→admit to NWing-TB isolation on 4-drugs, send home when stable with mask
- 4. Unstable/Village pt→admit to NWing-TB isolation on 4-drugs, send home when AFB x3 Neg (as before) and pt stable

Furthermore, as Xpert MTB/RIF can pick-up patients before they become contagious (unlike the AFB test), dx and tx will occur sooner meaning fewer in the community will be exposed. An additional advantage of this assay is the RIF component or the test for Rifampin resistance test which alerts for Multi-Drug resistance and enables immediate tx for MDR-TB.

NEGATIVE rapid sputum test result = No active pulmonary TB contagious or pre-contagious so a pneumonia can be treated inpatient or outpatient without concern for TB-isolation. Of course if a provider believes the test result may be a false-negative, TB-isolation can still be instituted.

Test Applications in the YK Delta

- More efficient TB medical care
- Improved Patient/Human Rights

The Xpert MTB/RIF assay gives a result within hours. Therefore, an otherwise stable pneumonia patient who does not have TB does not have to be "isolated" against his/her will for the safety of the community for 4-8 days while his 3 AFB sputums go through the AFB testing process in Anchorage to prove this.

Test Applications in the YK Delta

- More efficient TB medical care
- Improved Patient/Human Rights

- Improved Hospital and Community Safety

Since more actively TB-infected patients will be discovered with the Xpert MTB/RIF assay (and treated) before becoming contagious (unlike the AFB test), predictably fewer contagious TB patients will be circulating undiagnosed in the community and healthcare setting, thus a safer environment.

Test Applications in the YK Delta

- More efficient TB medical care
- Improved Patient/Human Rights
- Improved Hospital and Community Safety

- Reduced Healthcare Costs

Conservatively, the rapid TB test could potentially save YKHC at least $150,000 annually through;

- 1. Reduced unnecessary admissions of stable patients
- 2. Reduced number of admitted patients not requiring TB-isolation precautions services of healthcare and ancillary staff
- 3. Reduced RT services required
- 4. Fewer Alaska Air "Gold-Streak" lab specimens to the State Lab.
- 5. Other cost savings

Video: strung

Review of Lecture Objectives

- 1. Explain what Nobel Prize-winning technology is behind this test, how it works and why it is so accurate.
- 2. Understand the current TB diagnostic limitations, especially in Bush Alaska, and how Xpert MTB/RIF will transform them.
- 3. Make a case for improving Human Rights in Bush Alaska by implementing this test.
- 4. Understand why Alaska is so late in having access to this test while half the world has been using it for the past two years and especially, in which country?

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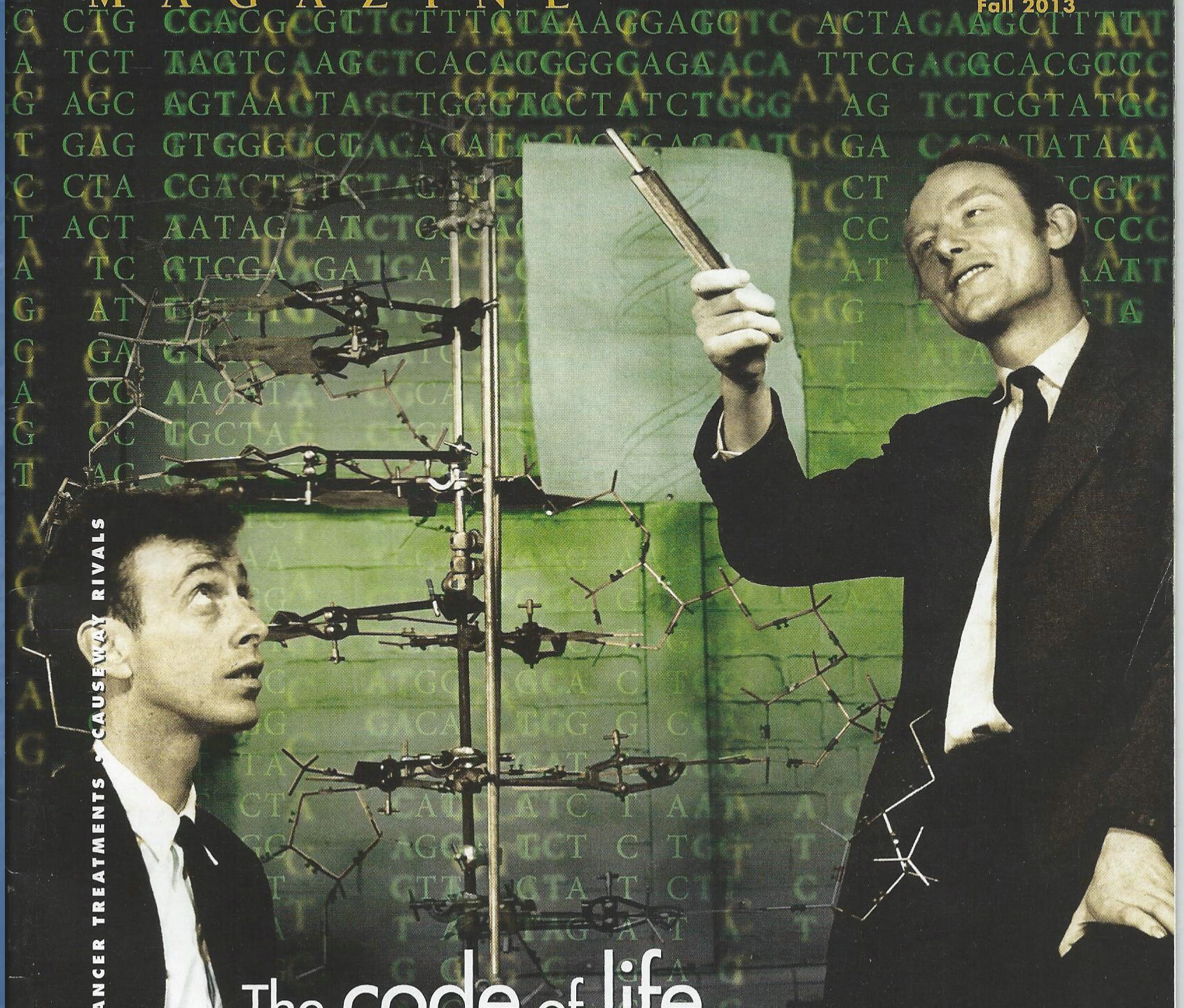

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The code of life