Democratizing Diagnostics: a Journey from Bench to Bedside

David H Persing, MD, PhD

Chief Medical and Technology Officer
Cepheid, Sunnyvale, CA, USA

Chief Scientific Officer
Danaher Diagnostics Platform

Consulting Professor of Pathology
Stanford University, Stanford, CA, USA

“The Machine that will help end TB”

Cohen, J. MIT Technology Review, Dec 2012

In November 2011, Jabu Ngcobo, 25, felt a pain in her side and went to the KwaMsane clinic, which resembles a trailer park.

“I was all along thinking I had MDR TB because my two brothers and my sister had it,” says Ngcobo.

“The Machine that will help end TB”

Ngcobo’s speedy diagnosis and recovery were made possible by a machine called a GeneXpert, which sits atop a counter inside one of the trailers….

…..and resembles a high-end espresso maker.

Cohen, J. MIT Technology Review, Dec 2012

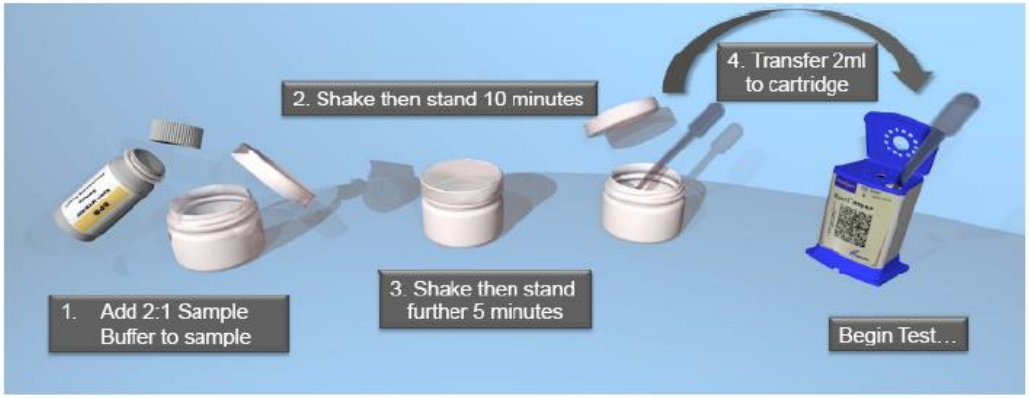
Not to be mistaken for a double shot decaf lemon twist latte…

- Sputum processing was the greatest technical challenge to Xpert test development
- Often bloody and full of inflammatory cells
- Cell-free DNA content makes it viscous and gravity-defying
- Variable number of bacilli requires large sample volume
- Requirement for inactivation

The Coffeemaker Analogy Taken Further

- Barrista: Can I have your order please?
- Customer: I'd like a double shot decaf latte with a twist of lemon
- Barrista: I'll put your order in right away….it will be available for pickup next Wednesday
- Why so long?
- We run our decaf double shot lattes in batches three times a week, and you just missed the cutoff time for the Monday run

(Paradigm) Shift Happens: Xpert MTB/Rif



Automated sample preparation

Amplification and detection

< 2 h

Xpert ™ MTB/Rif

Workflow

- fully automated with 1-step external sample prep.
- time-to-result < 2 h (walk away test)
- throughput: up to 1-48 tests / run
- no bio-safety cabinet
- closed system (no contamination risk)

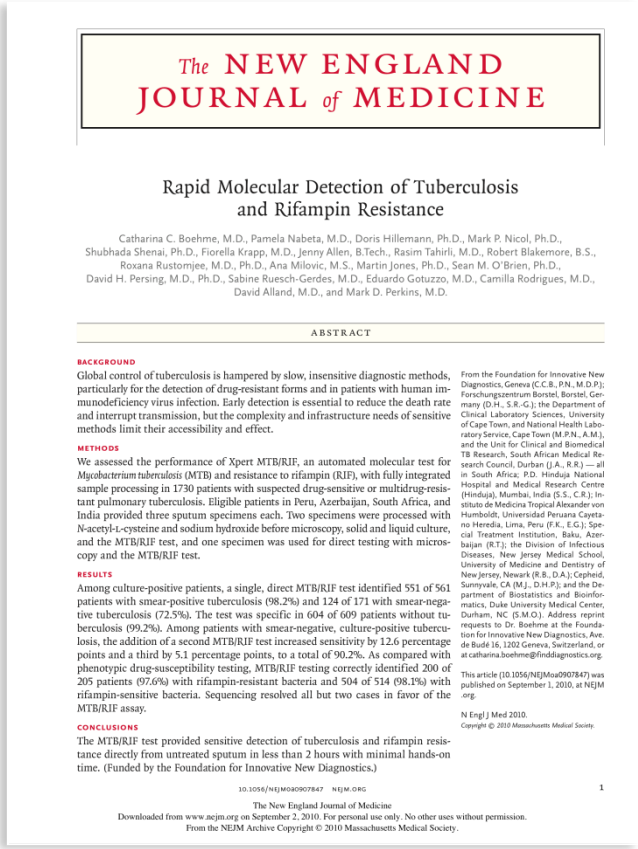
A technology platform for

- TB & Rif Resistance
- TB Quinolone resistance
- Potential for HIV viral load

Xpert® MTB/RIF

Rapid Molecular Detection of Tuberculosis and Rifampin Resistance'

Catharina Boehme, et al. **New England Journal of Medicine, 1 Sept, 2010**



The NEW ENGLAND JOURNAL of MEDICINE

Rapid Molecular Detection of Tuberculosis and Rifampin Resistance

Catharina C. Boehme, M.D., Pamela Nabeta, M.D., Doris Hillemann, Ph.D., Mark P. Nicol, Ph.D., Shubhada Shenai, Ph.D., Fiorella Krapp, M.D., Jenny Allen, B.Tech., Rasim Tahirli, M.D., Robert Blakemore, B.S., Roxana Rustomjee, M.D., Ph.D., Ana Milovic, M.S., Martin Jones, Ph.D., Sean M. O'Brien, Ph.D., David H. Persing, M.D., Ph.D., Sabine Ruesch-Gerdes, M.D., Eduardo Gotuzzo, M.D., Camilla Rodrigues, M.D., David Alland, M.D., and Mark D. Perkins, M.D.

ABSTRACT

BACKGROUND

Global control of tuberculosis is hampered by slow, insensitive diagnostic methods, particularly for the detection of drug-resistant forms and in patients with human immunodeficiency virus infection. Early detection is essential to reduce the death rate and interrupt transmission, but the complexity and infrastructure needs of sensitive methods limit their accessibility and effect.

METHODS

We assessed the performance of Xpert MTB/RIF, an automated molecular test for *Mycobacterium tuberculosis* (MTB) and resistance to rifampin (RIF), with fully integrated sample processing in 1730 patients with suspected drug-sensitive or multidrug-resistant pulmonary tuberculosis. Eligible patients in Peru, Azerbaijan, South Africa, and India provided three sputum specimens each. Two specimens were processed with *N*-acetyl-l-cysteine and sodium hydroxide before microscopy, solid and liquid culture, and the MTB/RIF test, and one specimen was used for direct testing with microscopy and the MTB/RIF test.

RESULTS

Among culture-positive patients, a single, direct MTB/RIF test identified 551 of 561 patients with smear-positive tuberculosis (98.2%) and 124 of 171 with smear-negative tuberculosis (72.5%). The test was specific in 604 of 609 patients without tuberculosis (99.2%). Among patients with smear-negative, culture-positive tuberculosis, the addition of a second MTB/RIF test increased sensitivity by 12.6 percentage points and a third by 5.1 percentage points, to a total of 90.2%. As compared with phenotypic drug-susceptibility testing, MTB/RIF testing correctly identified 200 of 205 patients (97.6%) with rifampin-resistant bacteria and 504 of 514 (98.1%) with rifampin-sensitive bacteria. Sequencing resolved all but two cases in favor of the MTB/RIF assay.

CONCLUSIONS

The MTB/RIF test provided sensitive detection of tuberculosis and rifampin resistance directly from untreated sputum in less than 2 hours with minimal hands-on time. (Funded by the Foundation for Innovative New Diagnostics.)

From the Foundation for Innovative New Diagnostics, Geneva (C.C.B., P.N., M.D.P.); Forschungszentrum Borstel, Borstel, Germany (D.H., S.R.-G.); the Department of Clinical Laboratory Sciences, University of Cape Town, and National Health Laboratory Service, Cape Town (M.P.N., A.M.), and the Unit for Clinical and Biomedical TB Research, South African Medical Research Council, Durban (J.A., R.R.) — all in South Africa; P.D. Hinduja National Hospital and Medical Research Centre (Hinduja), Mumbai, India (S.S., C.R.); Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru (F.K., E.G.); Special Treatment Institution, Baku, Azerbaijan (R.T.); the Division of Infectious Diseases, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark (R.B., D.A.); Cepheid, Sunnyvale, CA (M.J., D.H.P.); and the Department of Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC (S.M.O.). Address reprint requests to Dr. Boehme at the Foundation for Innovative New Diagnostics, Ave. de Budé 16, 1202 Geneva, Switzerland, or at catharina.boehme@finddiagnostics.org.

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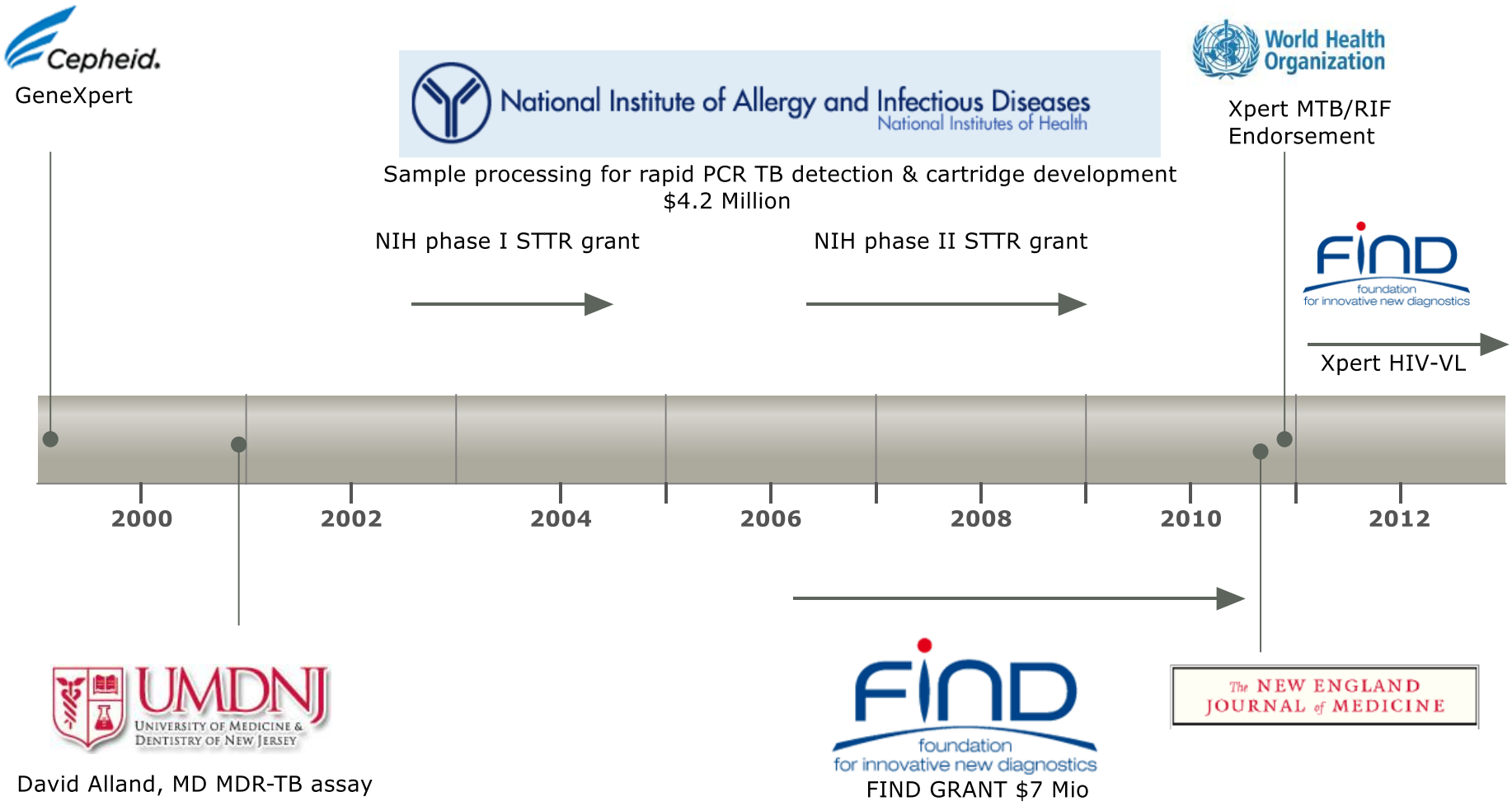
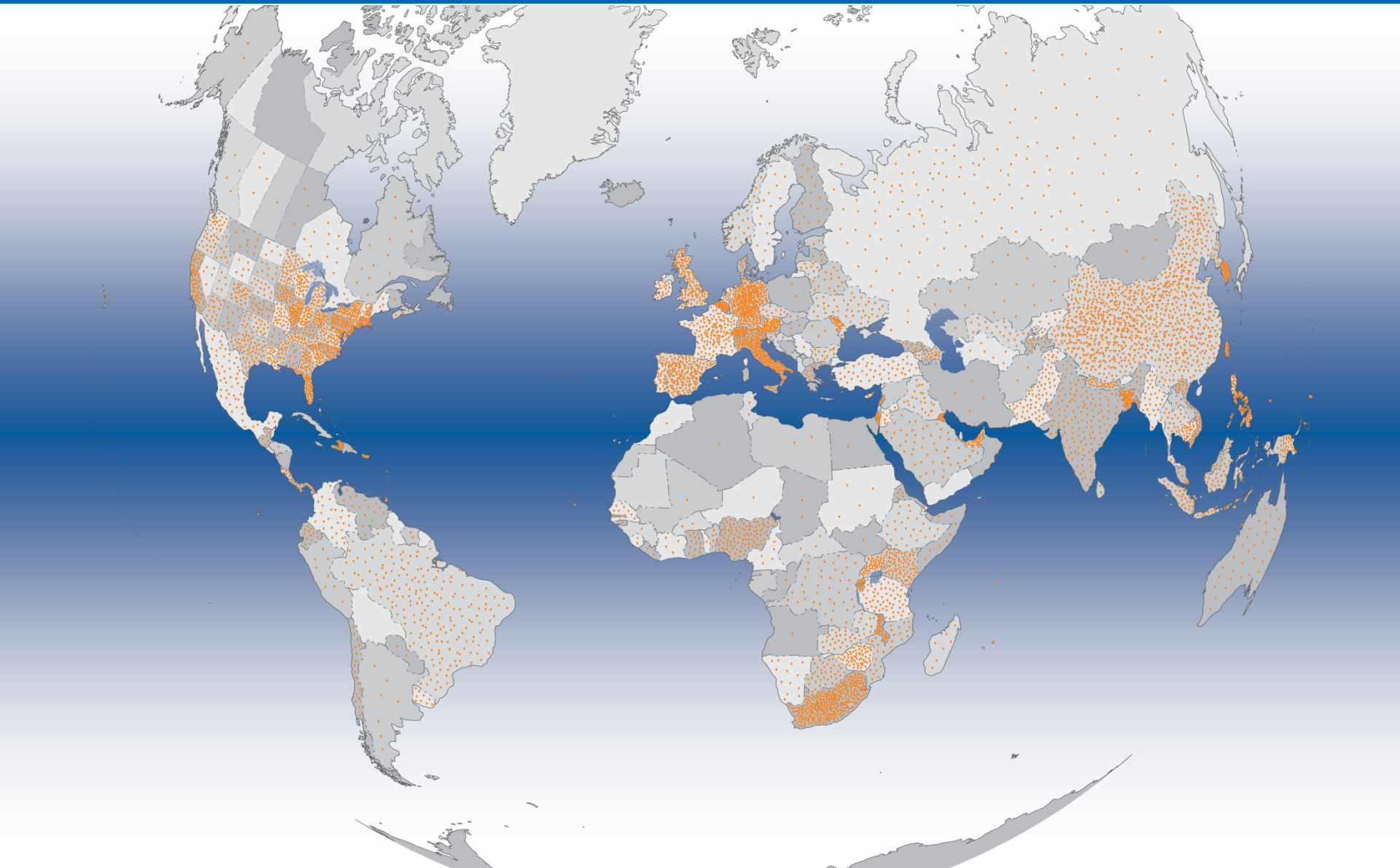
- Studied >1,700 Patients
  - Peru, Azerbaijan, South Africa and India
- Smear Positive Patients
  - 98.2% Sensitivity, 99.2% Specificity
- Smear Negative, Culture Positive Patients
  - 90.2% Sensitivity with Three Samples
  - 72.5% Sensitivity with One Sample
- Patients with Rifampin Resistance
  - 97.6% Sensitivity, 98.1% Specificity

Xpert MTB/RIF - A result of successful partnerships



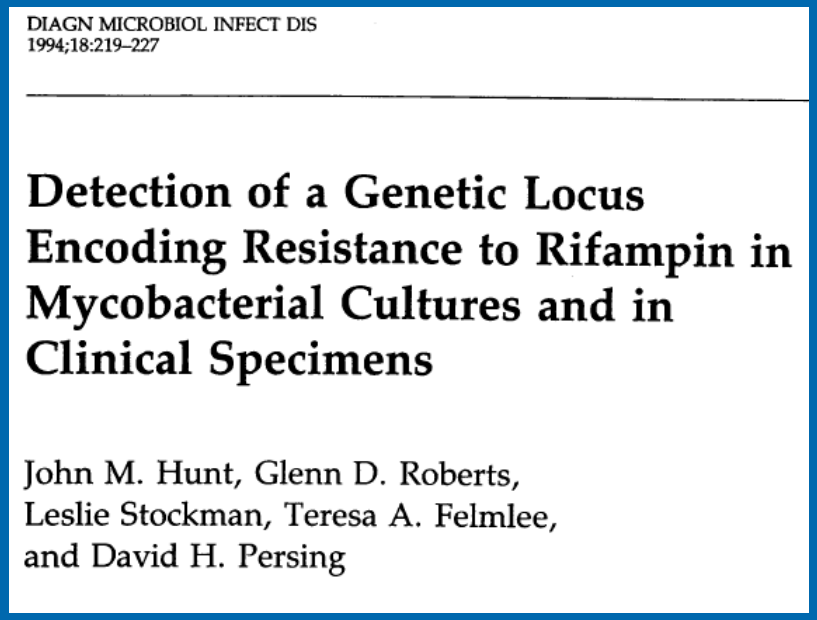
GeneXpert Placement Map

More than 15,000 systems



Going back a few years… to the bench

- In 1992, we asked if it would be possible to detect the TB organism and its drug resistance directly from a specimen
- The sequences for rifampin resistance in E. coli had just been published
- Two research fellows, Theresa Felmlee John Hunt used broad-range PCR to recover the sequences from TB and developed assays to detect resistance



DIAGN MICROBIOL INFECT DIS
1994;18:219–227

Detection of a Genetic Locus Encoding Resistance to Rifampin in Mycobacterial Cultures and in Clinical Specimens

John M. Hunt, Glenn D. Roberts,
Leslie Stockman, Teresa A. Felmlee,
and David H. Persing

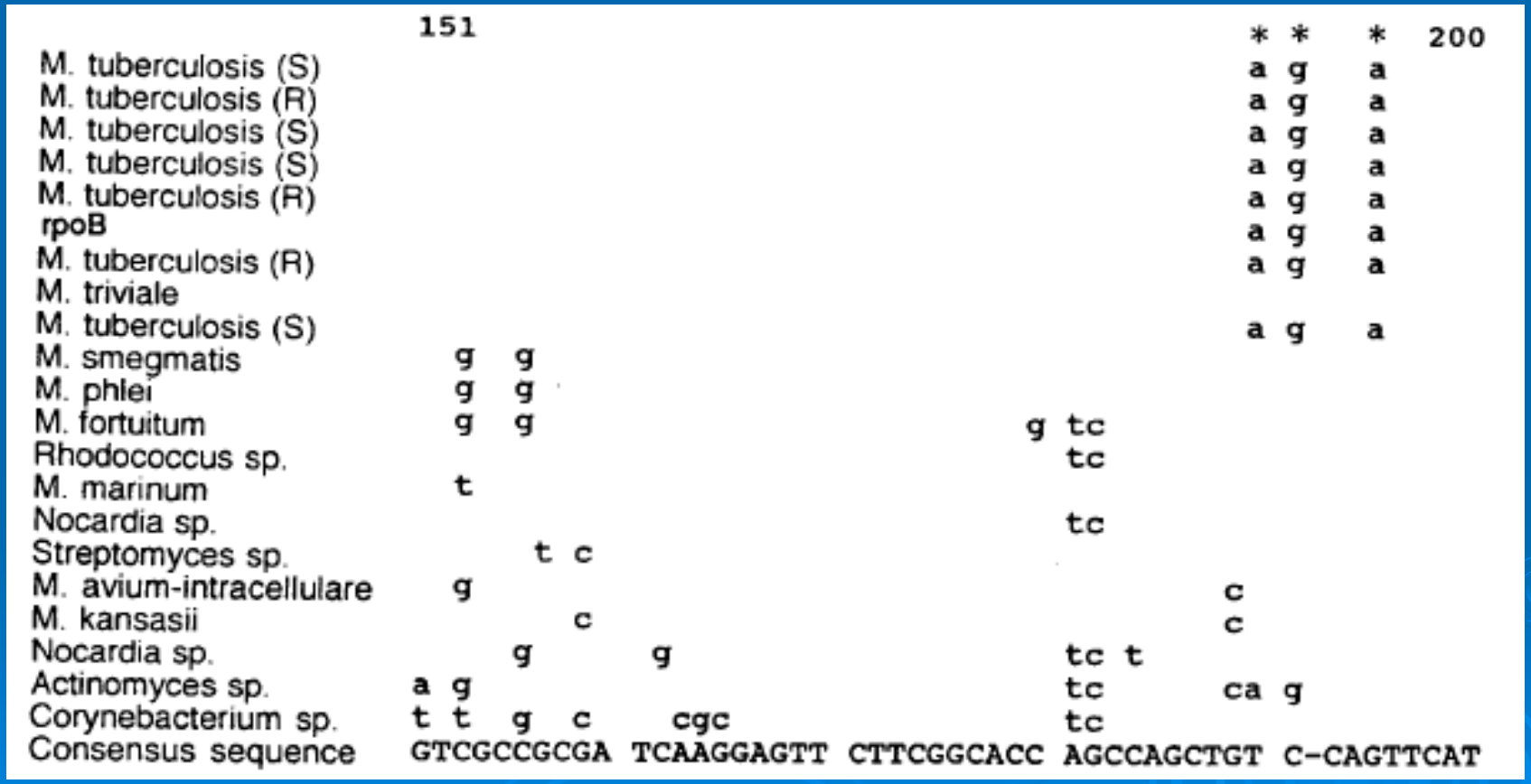
First demonstration of direct-specimen detection of TB along with simultaneous detection of drug resistance

Challenges that had to be overcome:

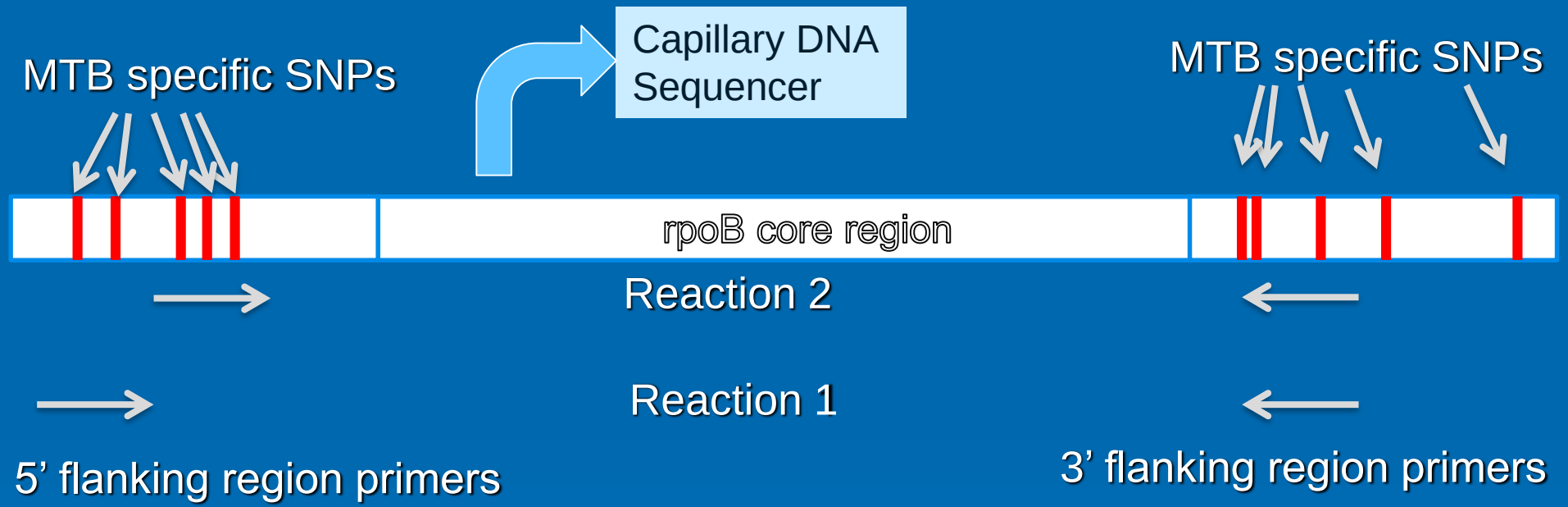
1) Non-specific amplification of rpoB from NTM
2) Contamination due to nested PCR procedure
3) Labor-intensive protocol requiring several days for completion
4) Humiliating rejection from a major journal

Sequence-specific Detection of MTB

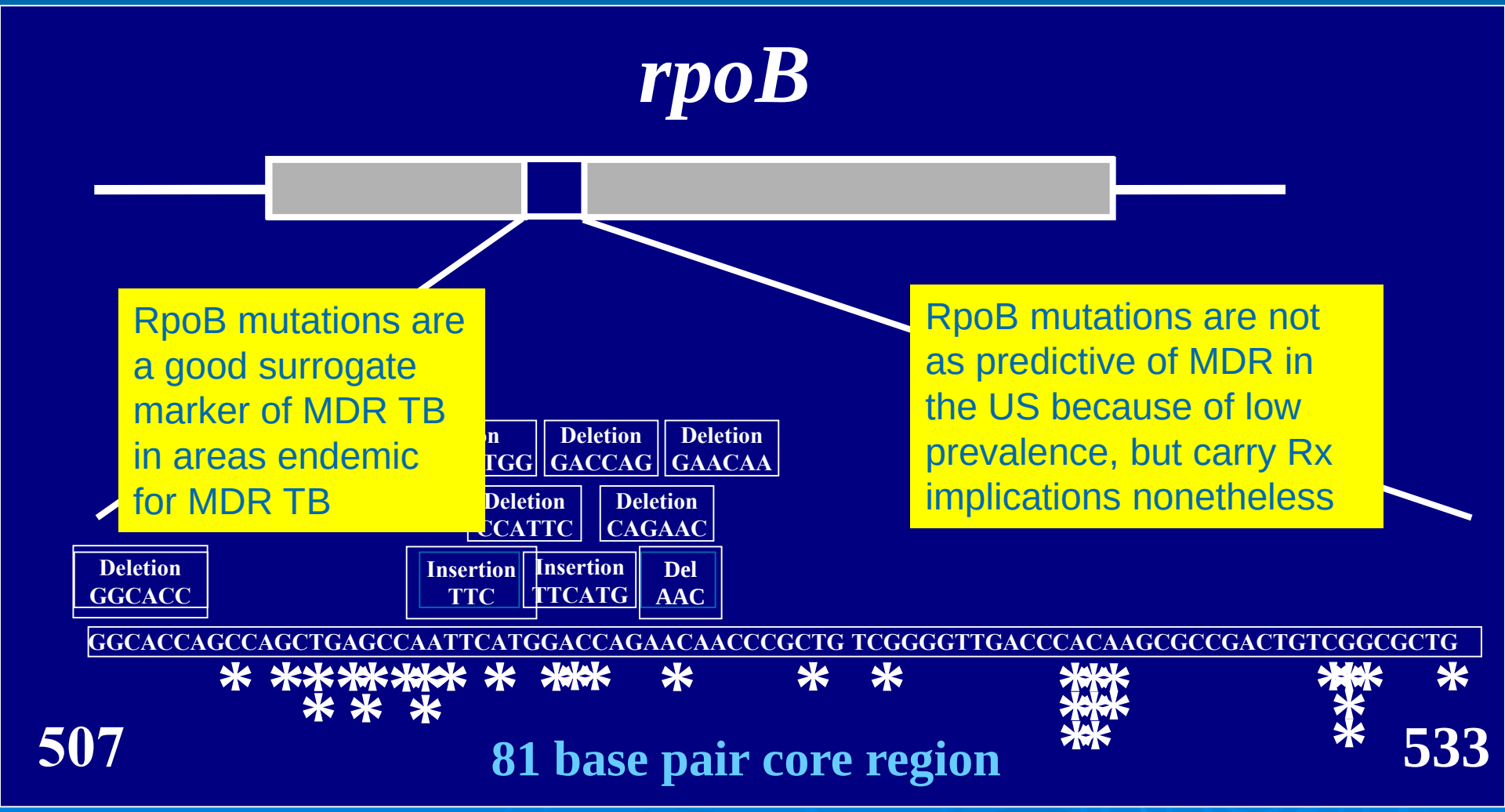
Hunt et al, DMID 1994 18:219



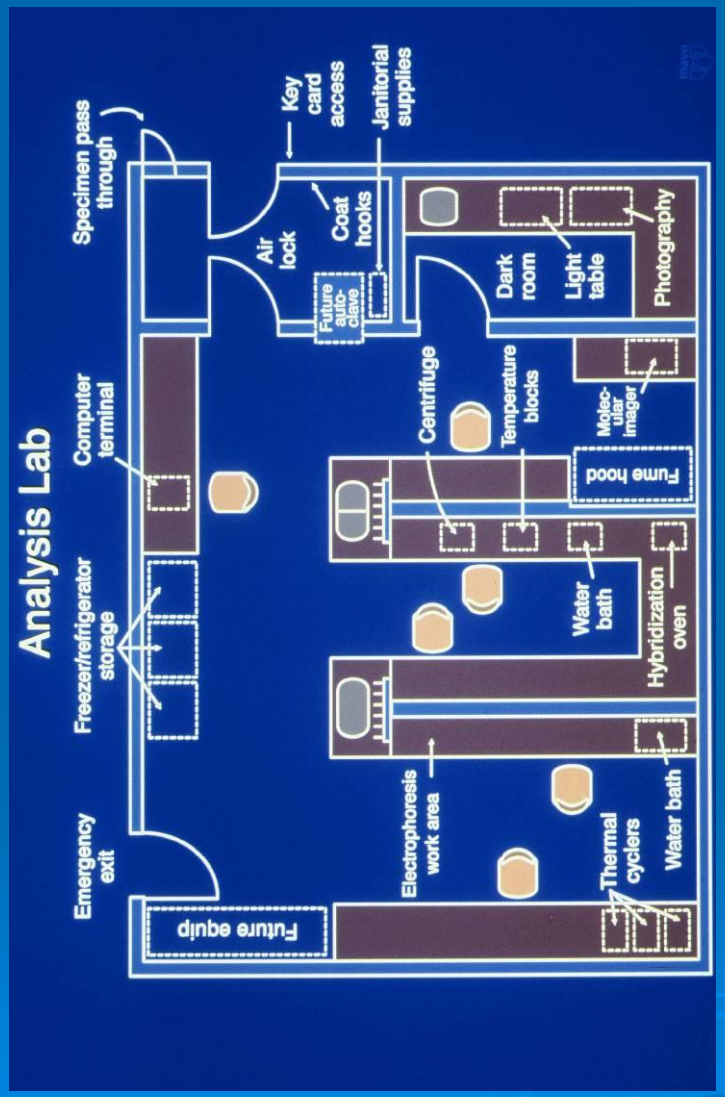
First generation MTB/rif: Mayo Clinic RpoB Assay Design



Genetic basis for Rifampin Resistance in *M. tuberculosis* has been known since the early 1990s



The “Dreaded” MTB Test at Mayo



- A herculean effort reserved only for emergencies
- Be careful of what you publish!
- 4000 Square Foot dedicated PCR lab
- 3 hermetically sealed rooms
- Specially trained medical technologists, 2 MD fellows, 1 PhD Director
- HEPA filtered air for each room (in and out)
- TAT about a week
- Nested protocol was prone to PCR product contamination, requiring multiple negative controls per run
- Back-up “clean rooms” in the event of contamination
- Estimated cost: hundreds of dollars
- Estimated charge would have been thousands

We used the test in cases like this:

- 50 year old diabetic man from Saudi royal family
- 20 year history of treatment for pulmonary TB
- Poorly compliant WRT TB and diabetes medication
- Not high-ranking enough to merit use of the family private jet….so….

Mayo Clinic Proceedings, March 1996, 71;221-229

MDR-TB case, continued

- 8 hour flight aboard NWA (First class, London to Minneapolis)
- Productive cough, no protective mask
- Sat next to person who turned out to be HIV-positive
- Arrived at Mayo Clinic Rochester with few medical records
- Exposed multiple medical and admissions personnel before TB history was learned
- Follow-up study ordered by Mayo and MDH

Mayo Clinic Proceedings, March 1996, 71;221-229

Exposed to MDR-TB:

- 3 physicians
- 2 interpreters
- 8 registration and admissions clerks
- 2 X-ray technologists
- Unknown number of other patients in waiting areas
- Public exposure in local Rochester hotel and several restaurants
- An entire planeload of NWA passengers

Mayo Clinic Proceedings, March 1996, 71;221-229

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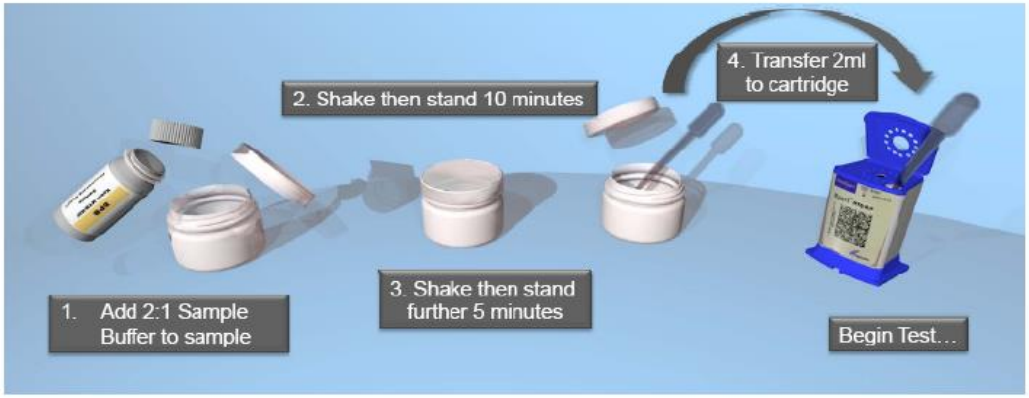

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A technology platform for

- TB & Rif Resistance
- TB Quinolone resistance
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What were the challenges in going from bench to bedside?

- Reference lab model could be applied selectively but results were not available quickly
- Methods required high level of skill and extreme attention to contamination control (due to nested PCR and sequencing of templates)…not democratizable
- The GeneXpert technology was a potential solution, but…..

What were the challenges in going from bench to bedside?

- Sputum processing turned out to be a big technical challenge (alcohol and lye became our best friends)
- Replacing sequencing by using molecular beacons to detect mutations was very finicky and required a lot of optimization
- Designing for assay stability under field conditions was difficult

What were the challenges in going from bench to bedside?

- System optics failed in dusty environments, and maintenance in remote locations has been a challenge
- Operator training and connectivity became an ongoing issue
- Import duties, distributor markups drove the cost from $10 to $70 and beyond

What did we do in response to these challenges?

- Undertook four design iterations to improve robustness, stability, and reliability
- Strived to make the test as foolproof as possible
- Operator training online via Wi-Fi for test operation and basic system maintenance
- Worked with governments and MOH and NGOs to reduce trade barriers and implementation costs

What did we do in response to these challenges?

- Automated cartridge manufacturing lines to reduce costs and improve reproducibility
- Duplicated manufacturing lines in Stockholm (also solved some import barrier issues)
- Vertically integrated the supply chain for critical materials

Final Comments

- Going from bench to bedside takes much longer than you might think
- Concepts developed in 1990s took 20 years to translate into something truly useful
- Fortunately, since the platforms are now developed, B2B cycle times will be much shorter
- “Doing well by doing good” is possible, but it is easier said than done

THANK YOU

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