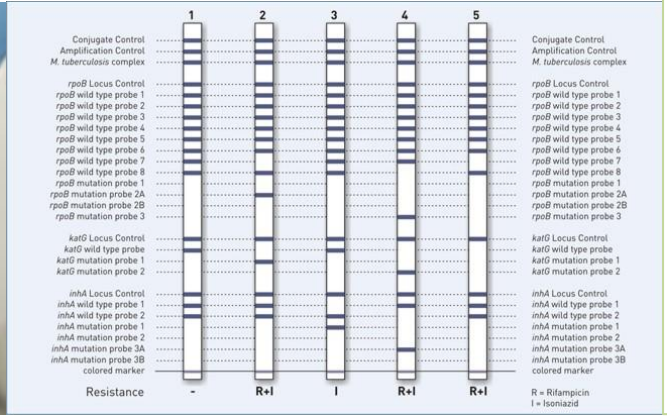


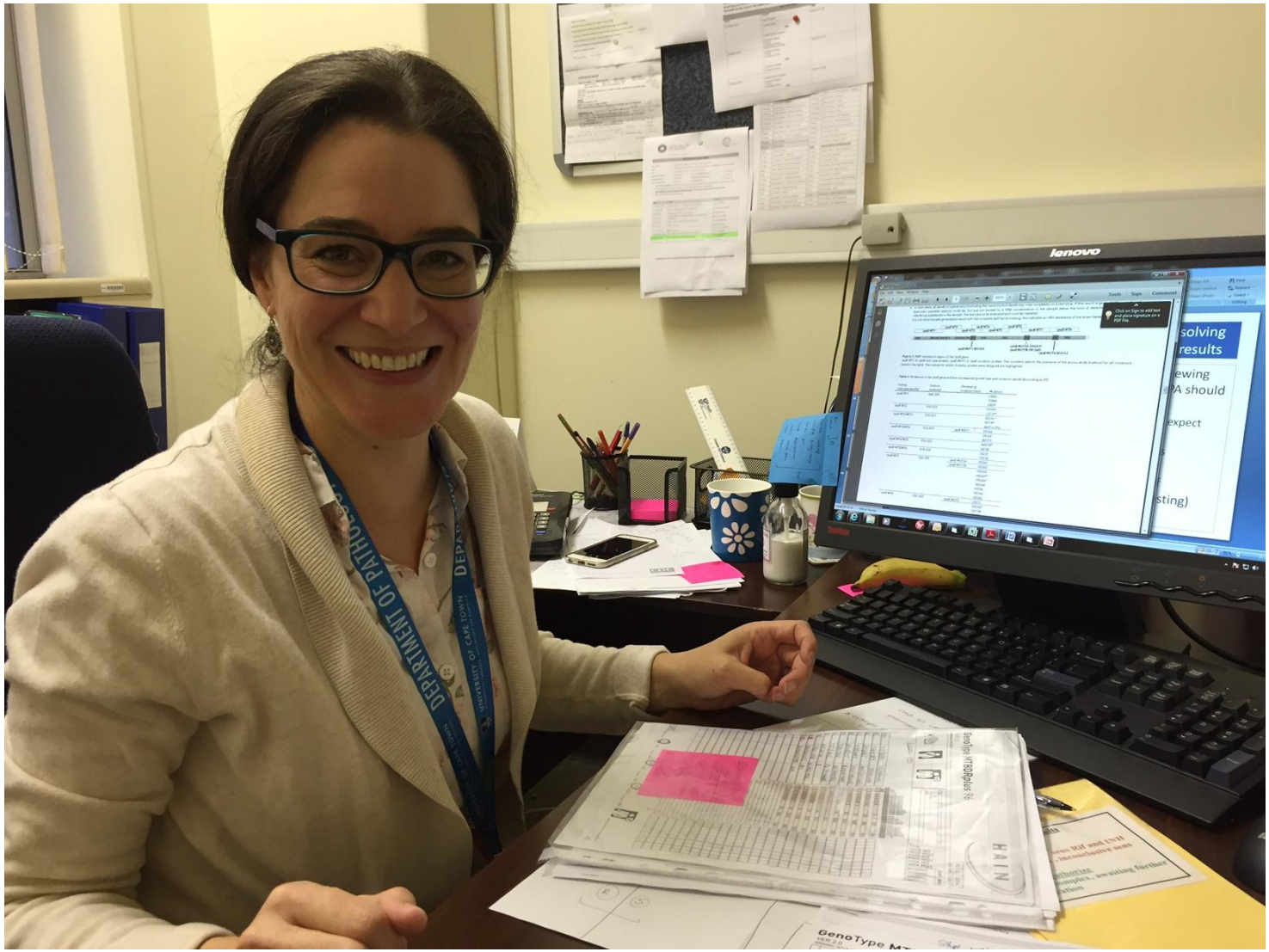
Management of discordant *Mycobacterium tuberculosis* resistance tests

Marc Mendelson

Division of Infectious Diseases & HIV Medicine

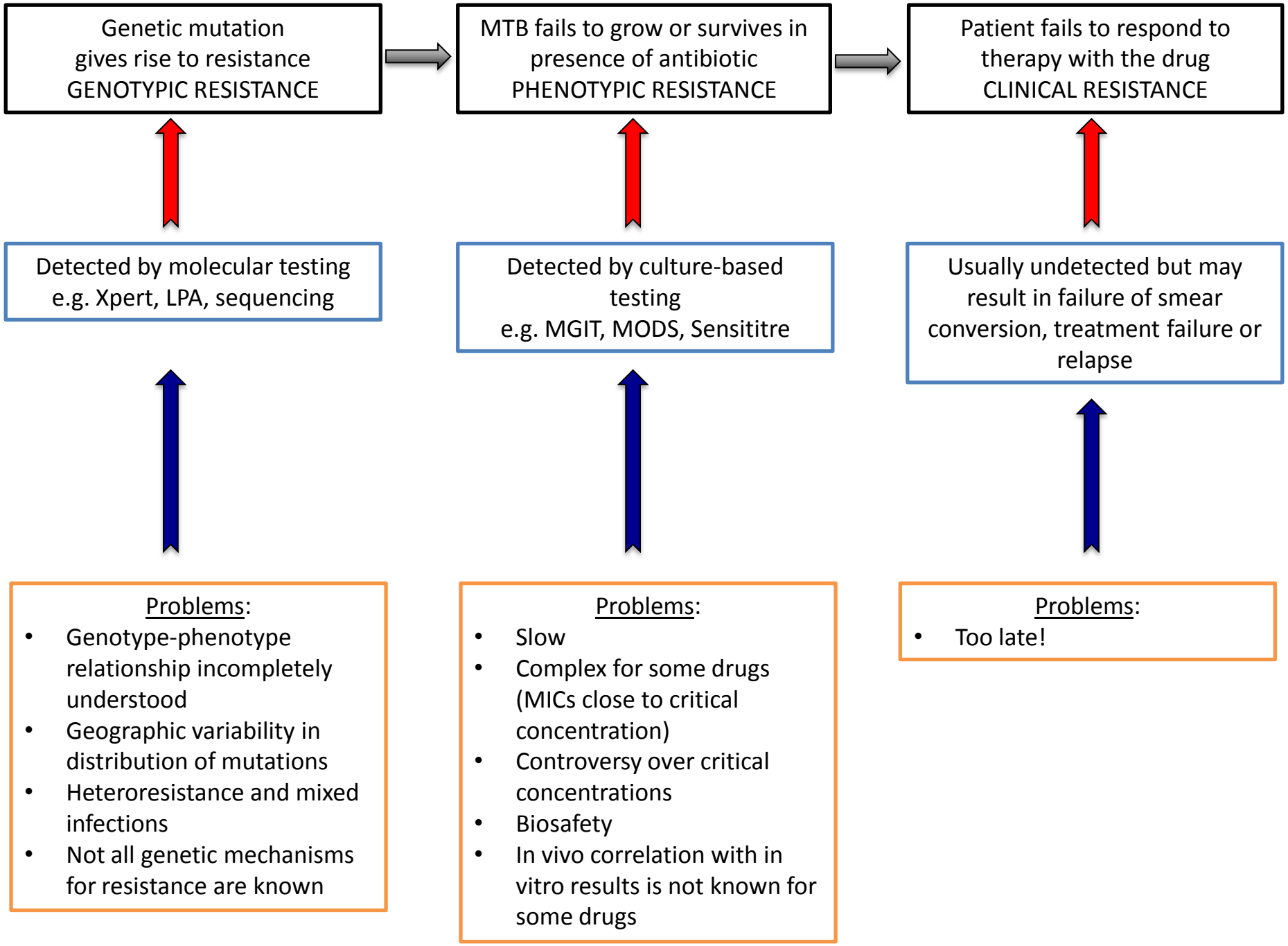
University of Cape Town

Credit, where credit's due



Discordance

- *"A state of non-harmony or non-agreement"*
- Non-agreement between
  - Different genotypic tests
  - Genotypic and phenotypic tests
  - 1 or more of genotypic and/or phenotypic tests and clinical response to treatment



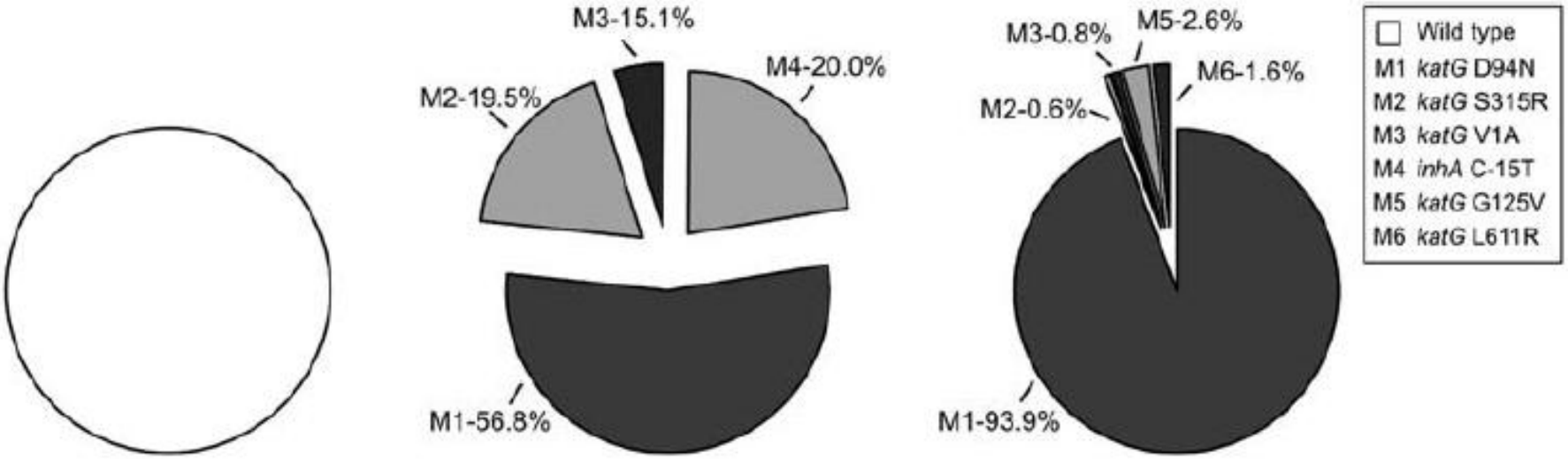
Slide courtesy of Mark Nicol

Heteroresistance

- Sensitive and resistant *M. tuberculosis* in a single clinical sample
- Resistance mutations arise from a single clone
- Spontaneous or driven by antibiotic selection pressure
- Picked up by LPA, molecular phenotyping (MIRU-VNTR) or by genome sequencing
- Assaying single samples doesn't allow us to define full extent of heteroresistance, nor the significance of various mutations

Dynamic Population Changes in *Mycobacterium tuberculosis* During Acquisition and Fixation of Drug Resistance in Patients

Gang Sun,[1,a] Tao Luo,[1,a] Chongguang Yang,[1] Xinran Dong,[2] Jing Li,[3] Yongqiang Zhu,[4] Huajun Zheng,[4] Weidong Tian,[2] Shengyue Wang,[4] Clifton E. BarryIII,[5] Jian Mei,[3] and Qian Gao[1]



Untreated

19 months therapy

4 INH mutations
3 in *katG*,
1 in *inhA* promoter

24 months therapy

Expansion of *katG* D94N
Suggesting superior fitness

J. Infect Dis 2012;206:1724-33

Mixed Populations

- Presence of drug-sensitive and drug-resistant populations of different clonality in the same person
- Driven by high rates of infection & re-infection in TB-endemic populations

Van Rie et al. Am J Resp Crit Care med 2005;172: 636-42
Shamputa et al. Respir Res 2006; 7:99

Identification of multiple *M. tuberculosis* infections in sputum samples

March 2000 to June 2002
(**HIV co-infection < 10 %**)

186 patients

Male 126 and Female 60

61 patients infected with Beijing strains

Male 40 and Female 21

Single infection

26 (43%) patients infected with a Beijing strain only

Male 19 and Female 7

Mixed infection

35 (57%) patients infected with a Beijing strain and a non-Beijing strain.

Male 21 and Female 14

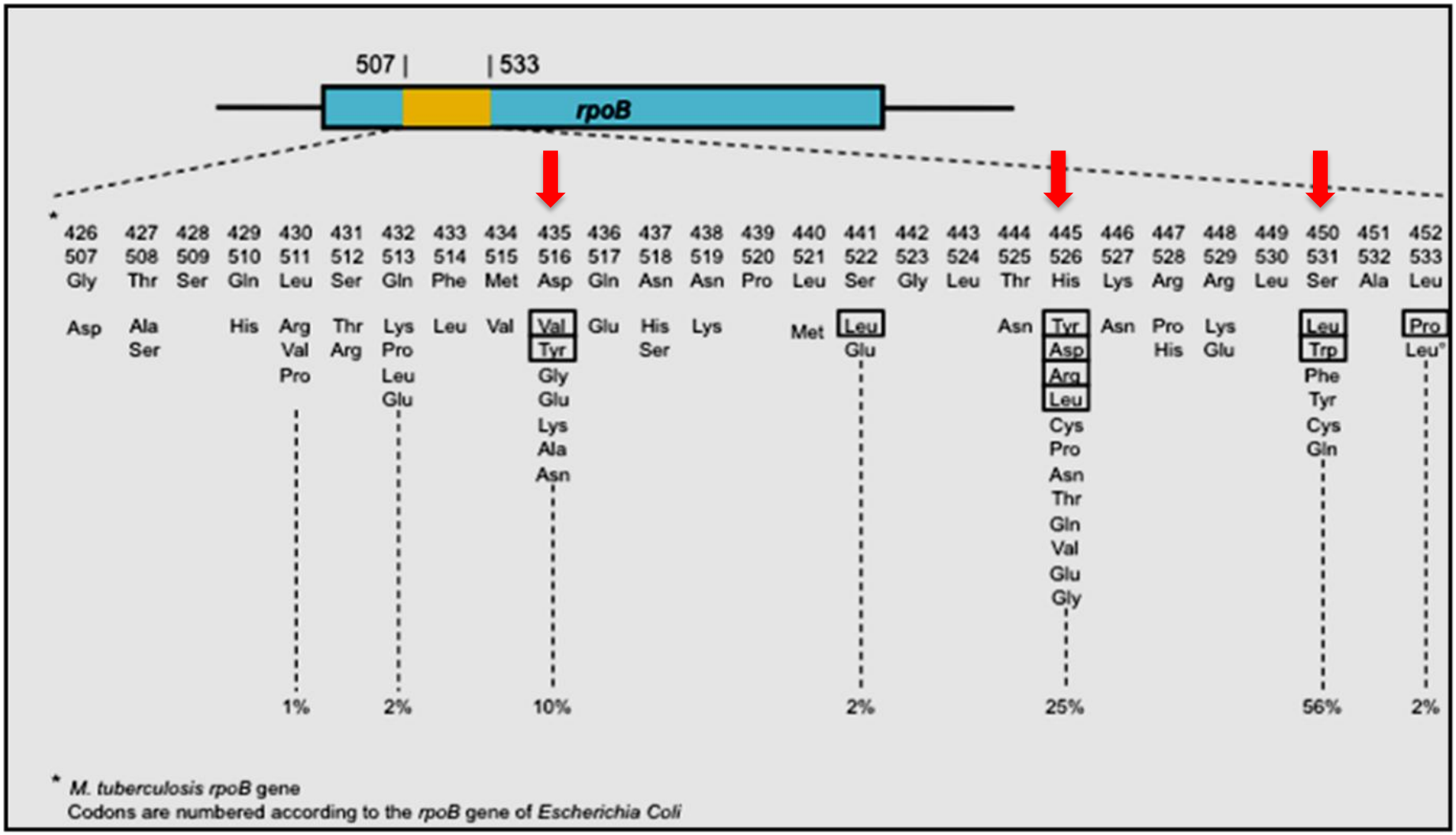
Warren, R. M., T. C. Victor, E. M. Streicher, M. Richardson, N. Beyers, N. C. van Pittius, and P. D. van Helden. 2004. Patients with active tuberculosis often have different strains in the same sputum specimen. *Am.J.Respir.Crit Care Med.* 169:610-614.

Slide courtesy of Paul van Helden

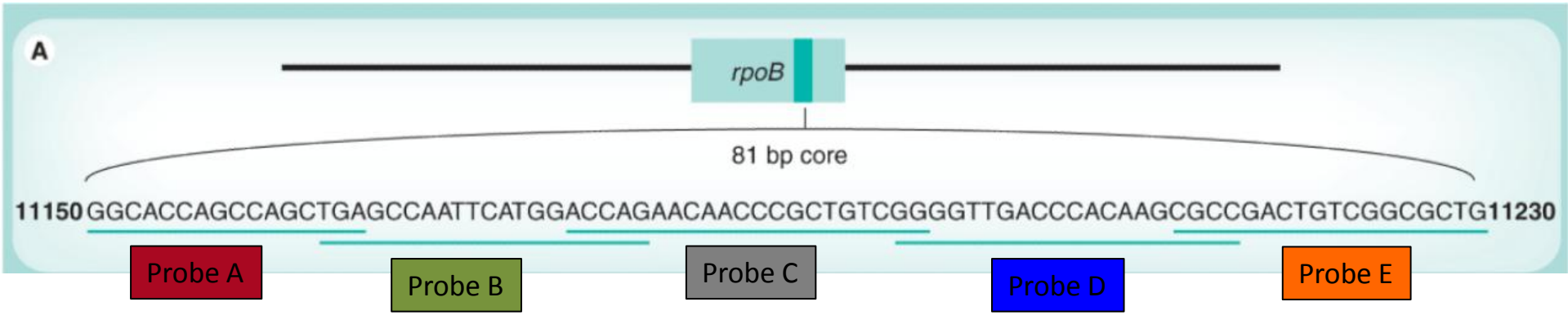
Rifampicin action & resistance

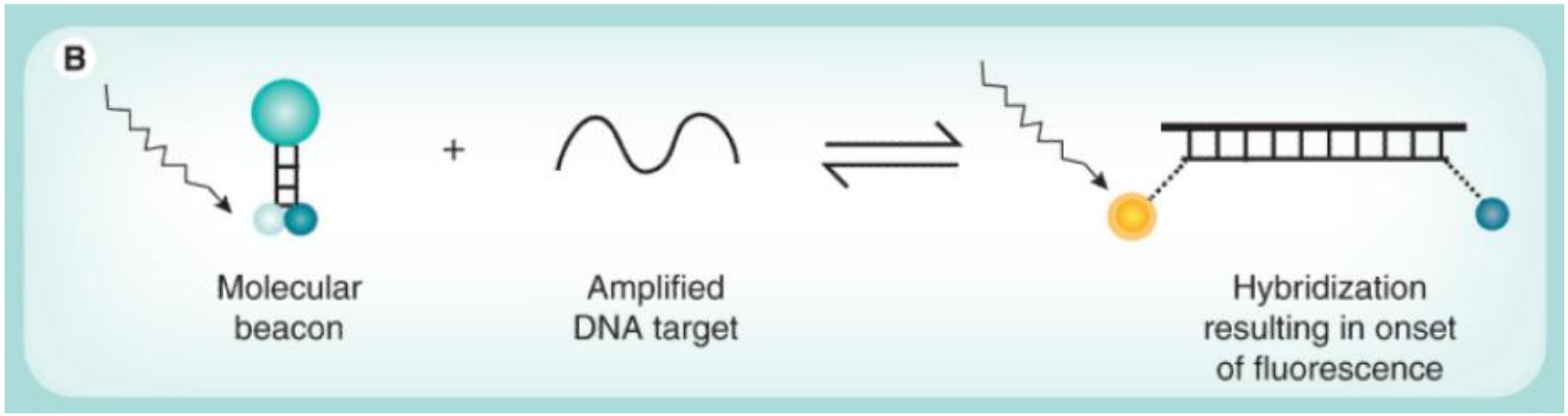
- Inhibits DNA synthesis by binding to RNA polymerase
- RNA polymerase is encoded by the *rpo*B gene
- 95% of rifampicin resistance due to SNP in the 81bp rifampicin resistance determining region (RRDR)
  - alters RNA pol structure, inhibiting rifampicin binding
- RRDR is the target for Xpert MTB/RIF and GenoType MTBDR*plus* line probe assay

Rifampicin Resistance Determining Region (RRDR)



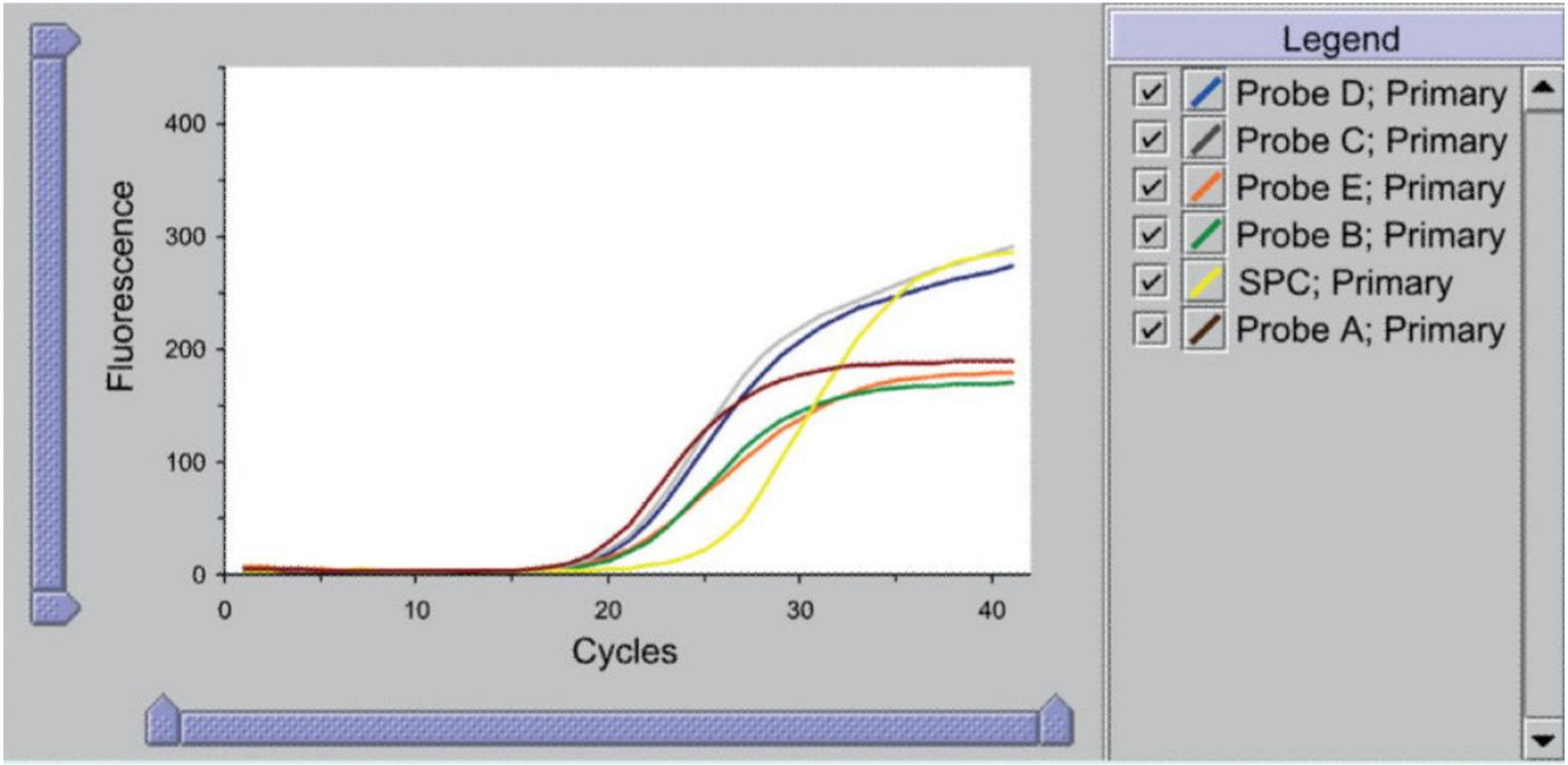
Xpert MTB/RIF Molecular Beacons





Lawn & Nicol. Future Microbiol. 2011 Sep; 6(9): 1067–1082.

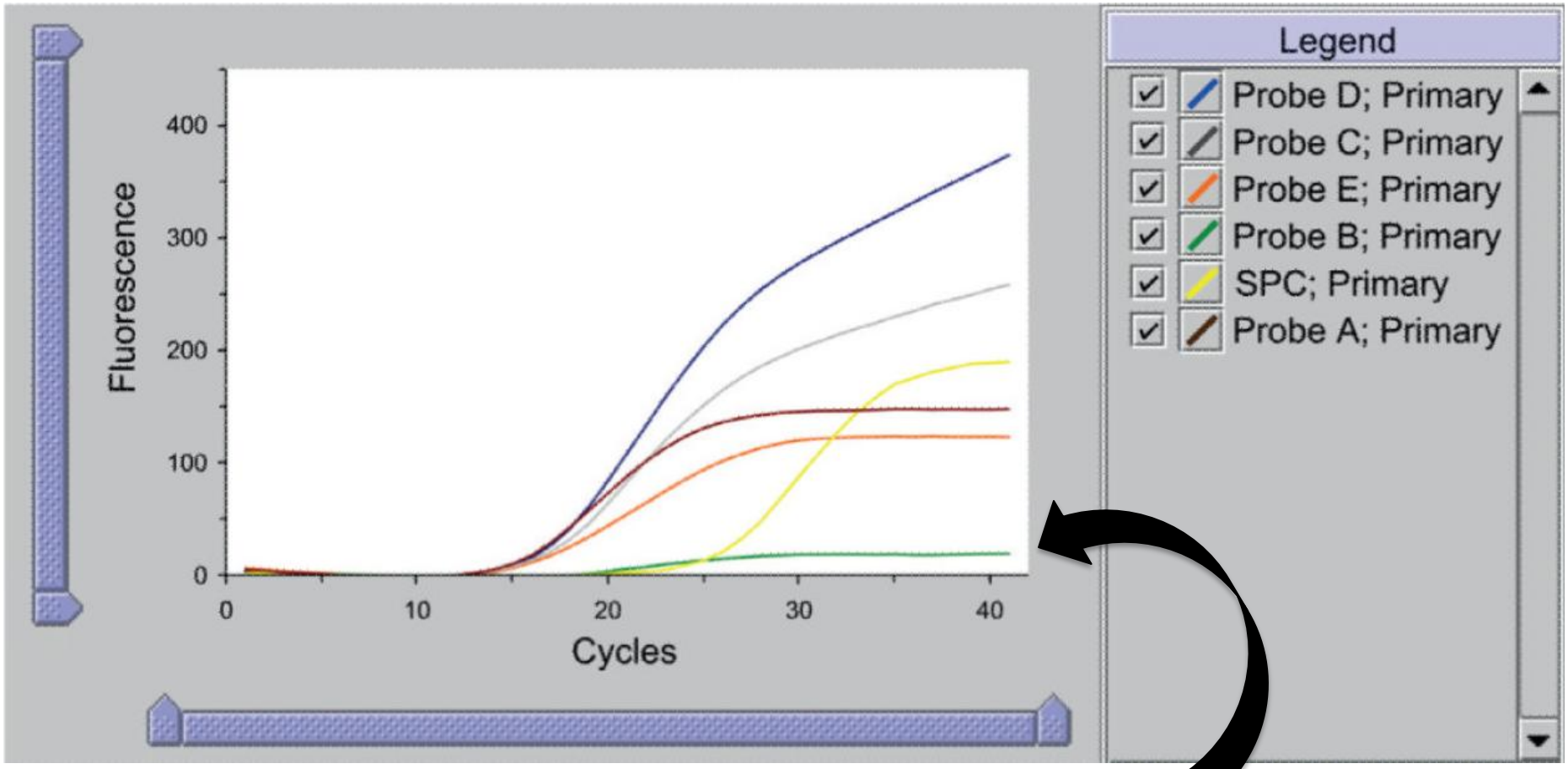
Rifampicin Sensitive Xpert



All 5 probes & *B. globigii* control amplify (fluoresce)

Lawn & Nicol. Future Microbiol. 2011 Sep; 6(9): 1067–1082.

Rifampicin Resistant Xpert



Probe B (green) fails to amplify

Lawn & Nicol. Future Microbiol. 2011 Sep; 6(9): 1067–1082.

Trouble shooting Xpert MTB/RIF: False-positive rifampicin resistance

- Procedural
  - preparation of specimen
  - delay in running the assay
  - air bubbles
- Very low Mycobacterial load
- Delay to reach cycle threshold (C_T) rather than dropout
  - C_T delay <4 sensitive
  - C_T delay >5 resistant
  - C_T delay 4.1 – 4.9 difficult to interpret
- Probe E involvement or >1 probe involved (D + E)
- Extra-pulmonary specimens

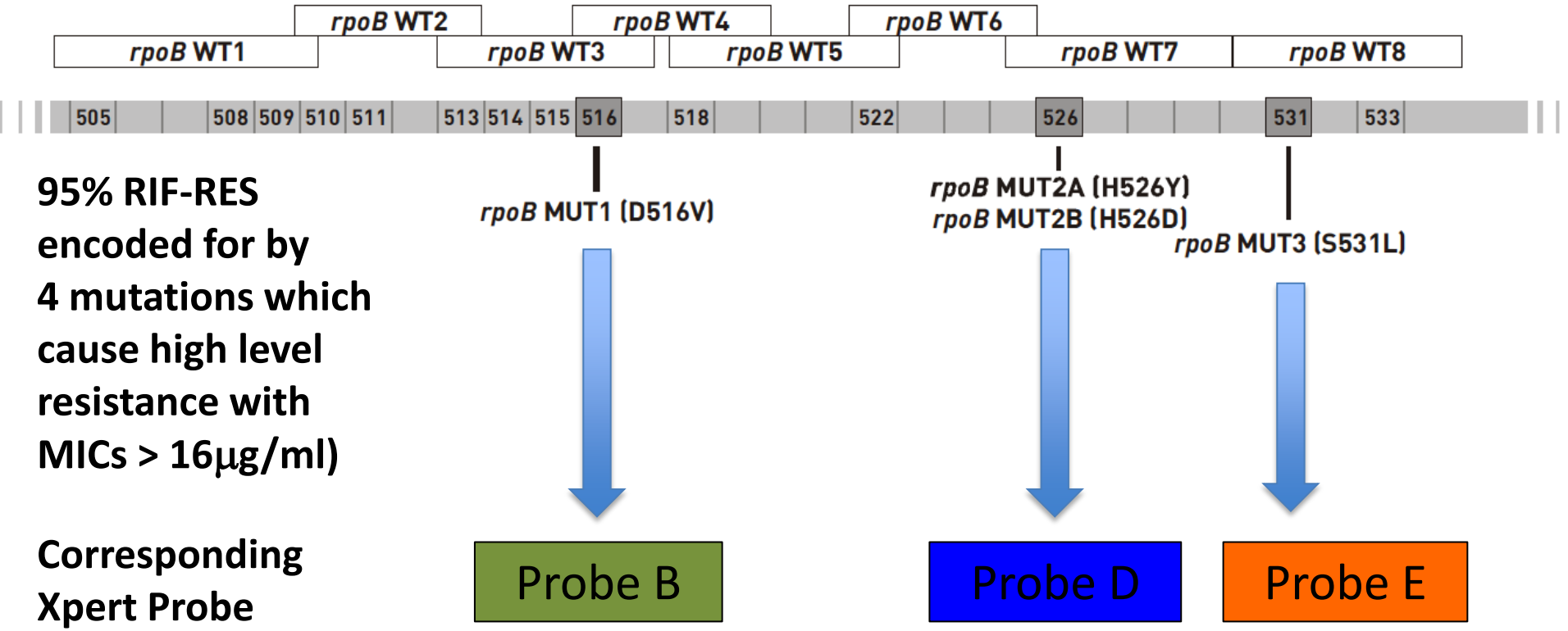
Ghebrekristos Y et al. & Berhanu R et al. Union TB conference, Cape Town, 2015

Trouble shooting Xpert – False-negative 'susceptible' in mixed infection or heteroresistance

- Need 65-100% resistant strain DNA to be picked up
- Resistant strains partially inhibiting hybridization, would only need small concentration of sensitive strain amplicon to boost probe signal into normal range
- More common in hyperendemic regions

Blakemore. JCM 2010;48:249-51

GenoType MTBDR*plus* version 2.0

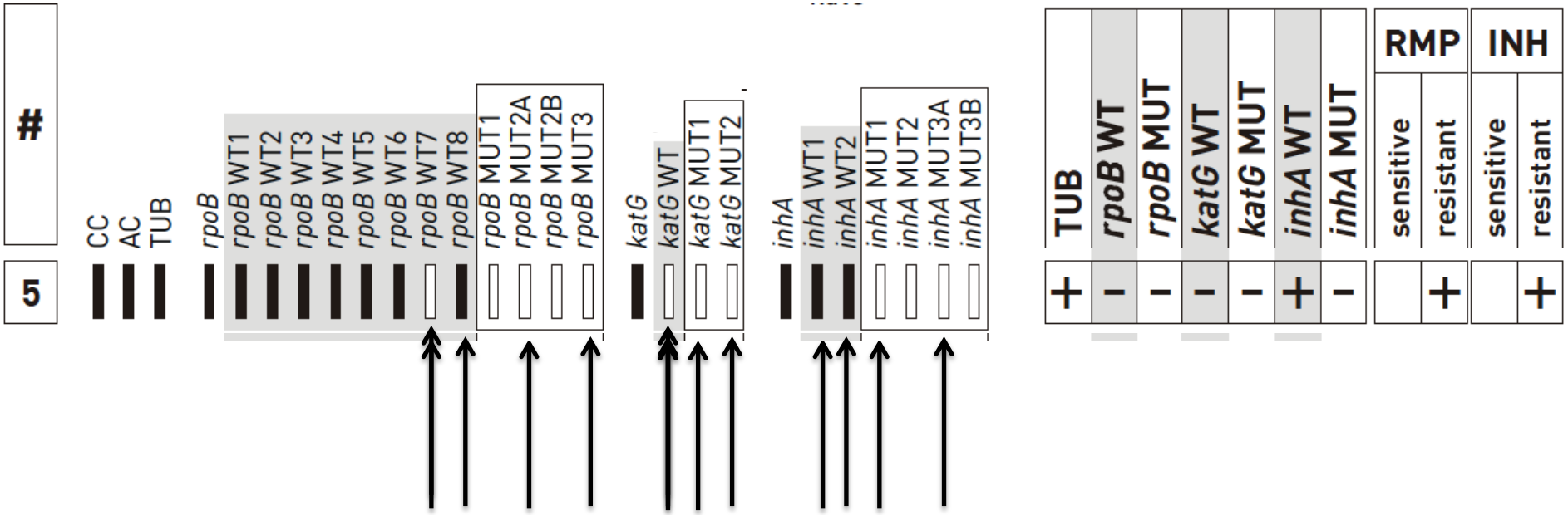


95% RIF-RES encoded for by 4 mutations which cause high level resistance with MICs > 16µg/ml)

Corresponding Xpert Probe

HAIN LIFESCIENCE

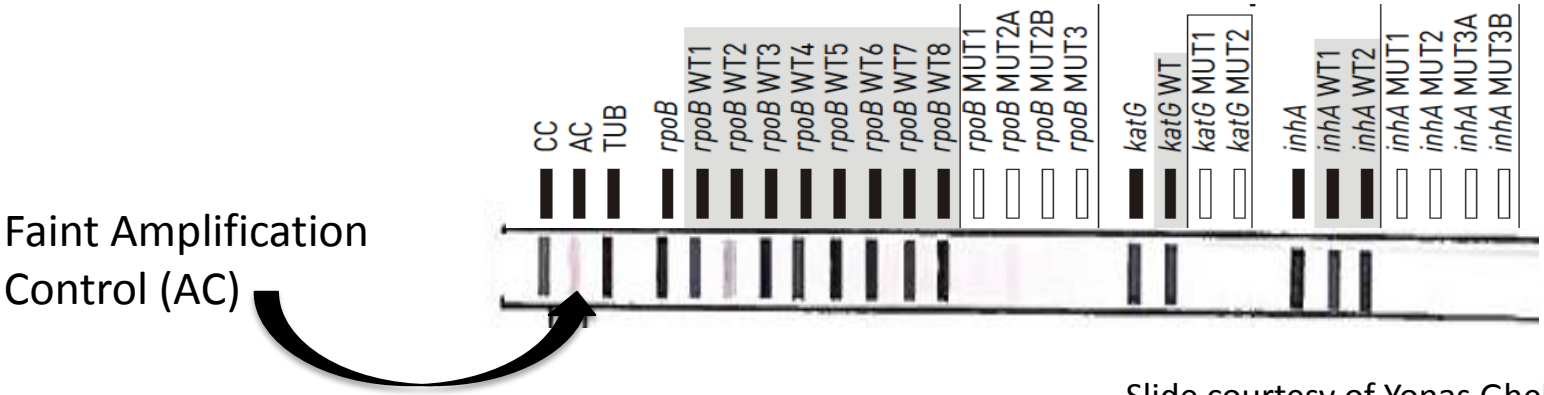
Examples of GenoType MTBDR*plus* Sensitive & Resistance profiles



HAIN LIFESCIENCE

Trouble shooting GenoType MTBDR*plus*

Error	False positive 'resistant'	False negative 'susceptible'
Procedural	Hybridization Bands too dark	Hybridization Bands too light
	Cross contamination leads to overcalling heteroresistance/mixed infections	Some mutations at very end of amplified sequence (L533P) can be missed (earlier version)
Interpretation	Subjective reading error or scanner error	



Slide courtesy of Yonas Ghebrekristos

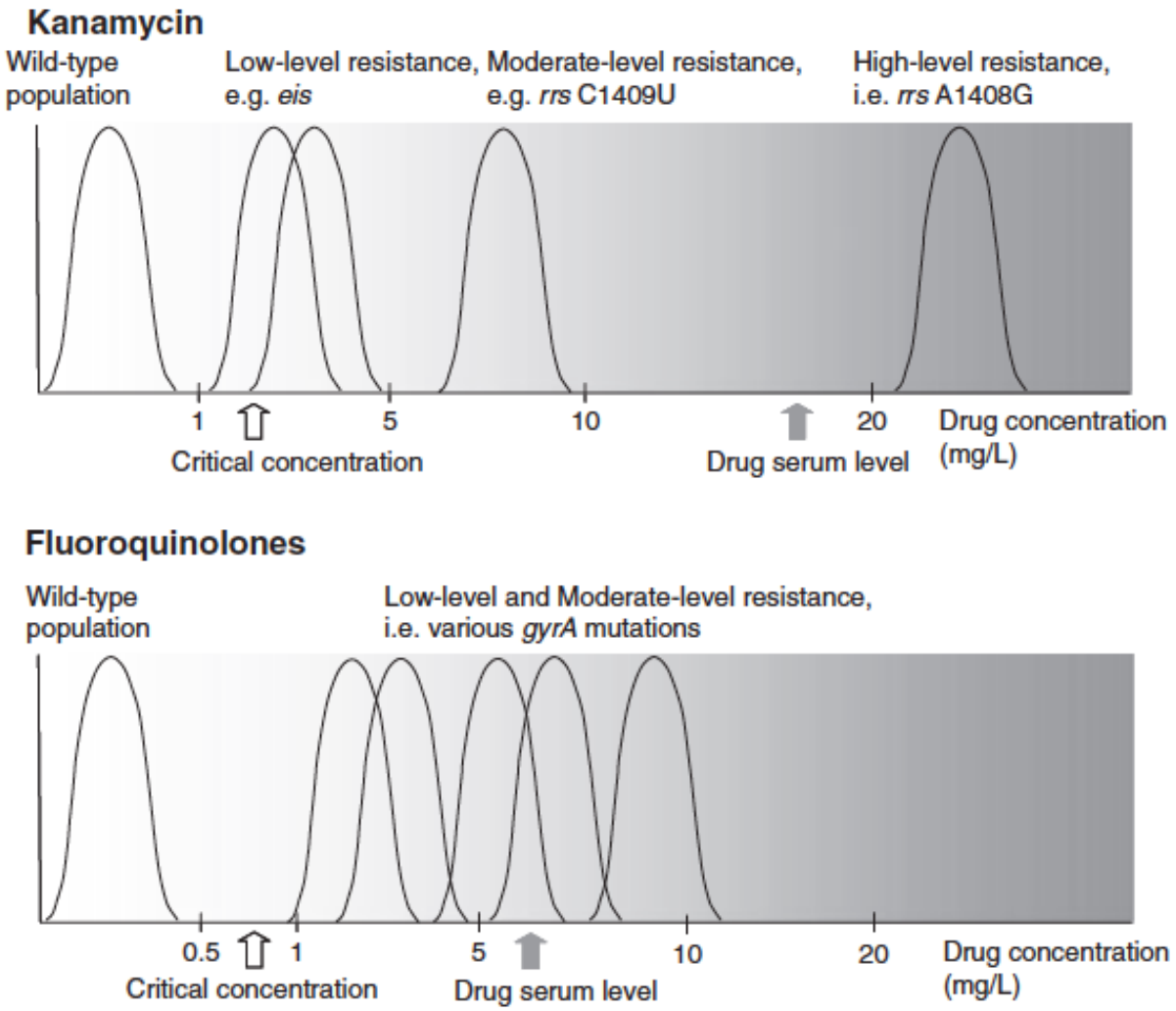
Culture–based DST

- Critical concentration of drug at which susceptible strains don't grow & resistant strains do
- Agar proportion method considered reference standard
- Future MIC testing would give more accurate information e.g. Sensititre

	1	2	3	4	5	6	7	8	9	10	11	12
A	OFL 32	MXF 8	RIF 16	AMI 16	STR 32	RFB 16	PAS 64	ETH 40	CYC 256	INH 4	KAN 40	EMB 32
B	OFL 16	MXF 4	RIF 8	AMI 8	STR 16	RFB 8	PAS 32	ETH 20	CYC 128	INH 2	KAN 20	EMB 16
C	OFL 8	MXF 2	RIF 4	AMI 4	STR 8	RFB 4	PAS 16	ETH 10	CYC 64	INH 1	KAN 10	EMB 8
D	OFL 4	MXF 1	RIF 2	AMI 2	STR 4	RFB 2	PAS 8	ETH 5	CYC 32	INH 0.5	KAN 5	EMB 4
E	OFL 2	MXF 0.5	RIF 1	AMI 1	STR 2	RFB 1	PAS 4	ETH 2.5	CYC 16	INH 0.25	KAN 2.5	EMB 2
F	OFL 1	MXF 0.25	RIF 0.5	AMI 0.5	STR 1	RFB 0.5	PAS 2	ETH 1.2	CYC 8	INH 0.12	KAN 1.2	EMB 1
G	OFL 0.5	MXF 0.12	RIF 0.25	AMI 0.25	STR 0.5	RFB 0.25	PAS 1	ETH 0.6	CYC 4	INH 0.06	KAN 0.6	EMB 0.5
H	OFL 0.25	MXF 0.06	RIF 0.12	AMI 0.12	STR 0.25	RFB 0.12	PAS 0.5	ETH 0.3	CYC 2	INH 0.03	POS	POS

Lee J. AAC 2014;58(1):11

Principle of culture-based DST

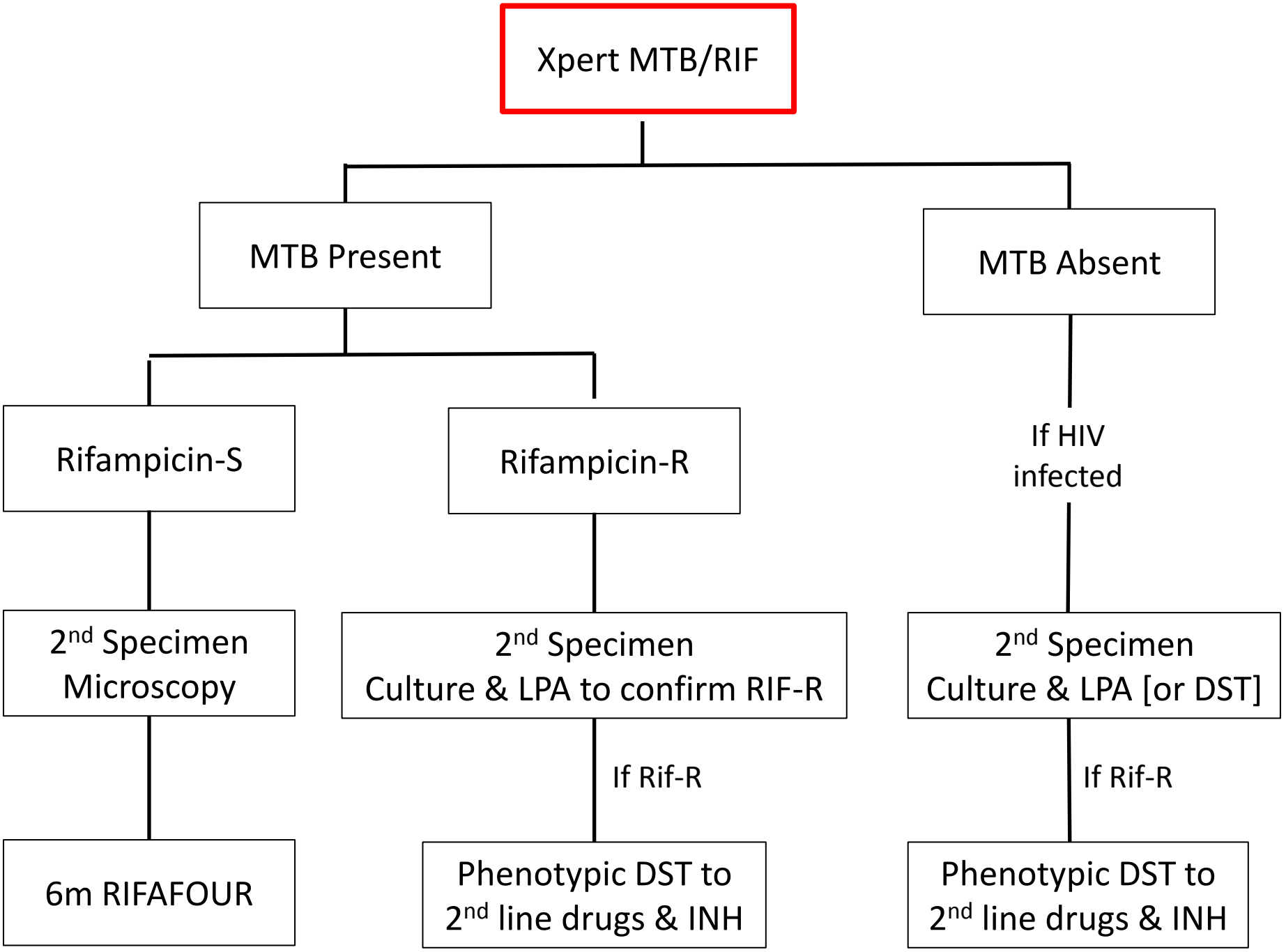


Bottger. Clin Microbiol Infect 2011, 17: 1128-34

Trouble shooting phenotypic DST

False Resistance	False Sensitive
Too low critical concentration of drug or loss of antibiotic potency	Too high critical concentration drug
Inoculum too high	Inoculum too low
Contamination with either NTM or DR-TB	

National algorithm for diagnosis of pulmonary tuberculosis


Xpert MTB/RIF
MTB Present
MTB Absent
Rifampicin-S
Rifampicin-R
If HIV infected
2nd Specimen Microscopy
2nd Specimen Culture & LPA to confirm RIF-R
2nd Specimen Culture & LPA [or DST]
If Rif-R
If Rif-R
6m RIFAFOUR
Phenotypic DST to 2nd line drugs & INH
Phenotypic DST to 2nd line drugs & INH

Concordant Rifampicin Resistance

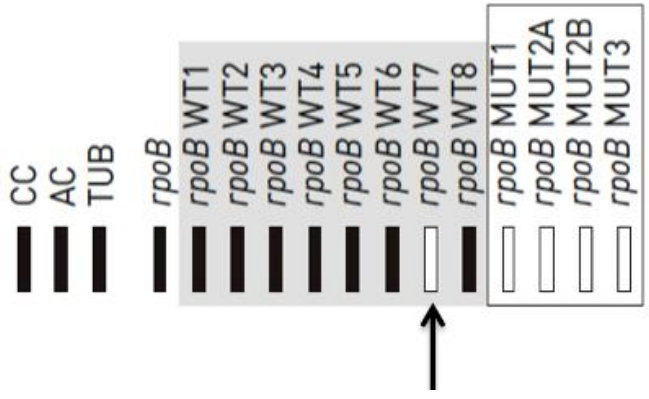
XPERT	LPA	Pheno -DST	Scenario	Explanation
R	R	R	>95% cases	>90% of cases *rpoB* mutations are high level RIF resistant (MICs>16ug/ml) & detected by MGIT DST at a critical concentration of 1ug/ml These are S531L, H526Y, H526D, D516V (all specifically detected by LPA)

Slide courtesy of N Beylis

Discordant Rifampicin Results (1)

XPERT-R : LPA-R : Pheno-DST-S

Scenario	Explanation
Uncommon Affects <5-10% of all *rpoB* mutations in the RRDR	• Disputed *rpoB* mutations in RRDR may be detected by Xpert & LPA • Effect on DST varies - low level resistance or susceptible, depending on SNP • Needs confirmation by rpoB sequencing and MIC testing



Adapted from slide by N Beylis

Discordant Rifampicin Results (2)
XPERT-R : LPA-S : Pheno-DST-S/R

Scenario	Explanation
Uncommon but increasingly recognized	• False Xpert-R or false LPA-S must be decided by *rpoB* sequencing • GSH/Greenpoint study of 100 patients over 12m* - XPERT-R was FALSE in 77% (no *rpoB* mutation) - LPA-S was FALSE in 23% • Heteroresistance or mixed population
If XPERT-R is real	• Phenotypic DST result depends on whether the *rpoB* mutation confers high level resistance (Pheno DST-R) or low level resistance (Pheno-DST-S/R)

*Ghebrekristos Y et al. Union TB conference, Cape Town, 2015

Adapted from slide by N Beylis

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Fax: 021 404 4105
Practice No: 5200296

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Groote Schuur
7935

Page 1

Labno :

Patient
Address

Patient ID
Age(Sex)

Ref Dr
Clinic
Patient #
Taken 12/12/13 10:00 Regd 14/01/14 07
Report 13/03/15 10:13

1. Xpert: RIF-R

2. LPA: RIF-S

LABORATORY REPORT (COPY)

Specimen Retreatment suspect 0 months
Tests ordered TB Cult,TB PCR,TB Sens L1,Add commnt
Tests referred TB Cult,TB PCR

3. Phenotypic DST: RIF-S

Antimicrobial sensitivity

Agent	Result	Agent	Result
ISONIAZID	SENSITIVE	RIFAMPICIN	SENSITIVE
STREPTOMYCIN	not done	ETHAMBUTOL	not done

ADDITIONAL COMMENTS *Amended*

Authorised by : Dr E Hoosien Registrar Test(s): TB Sens L1
Automatically reviewed by computer Test(s): Add commnt

--- End of Laboratory Report ---

False Xpert-R & True LPA-S + Pheno-DST-S?

V

True Xpert-R & False LPA-S + Pheno-DST-S?

TRUE resistant Xpert

FALSE susceptible LPA

phenotypic result?

Sequencing: L533P mutation detected (not picked up on LPA on previous versions)

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Page 1

Labno :

Patient
Address

1. Xpert: RIF-R

2. LPA: RIF-S

Patient II
Age(Sex)

Ref Dr
Clinic
Patient #
Taken 12/12/13 10:00 Regd 14/01/14 07
Report 13/03/15 10:13

LABORATORY REPORT (COPY)

Specimen Retreatment suspect 0 months
Tests ordered TB Cult,TB PCR,TB Sens L1,Add commnt
Tests referred TB Cult,TB PCR

3. Pheontypic DST: RIF-S

Antimicrobial sensitivity

Agent	Result	Agent	Result
ISONIAZID	SENSITIVE	RIFAMPICIN	SENSITIVE
STREPTOMYCIN	not done	ETHAMBUTOL	not done

ADDITIONAL COMMENTS *Amended*

Authorised by : Dr E Hoosien Registrar Test(s): TB Sens L1
Automatically reviewed by computer Test(s): Add commnt

--- End of Laboratory Report ---

Sequencing: No mutation detected

False Xpert-R & True LPA-S + Pheno-DST-S?

V

True Xpert-R & False LPA-S + Pheno-DST-S?

FALSE resistant Xpert

True susceptible LPA

True Phenotypic-DST

Discordant Rifampicin Results (3)
XPERT-S : LPA-R : Pheno-DST-S/R

Scenario	Explanation
Uncommon as if Xpert-S, algorithm only allows 2nd specimen for microscopy, not LPA	• False Xpert-S / false LPA-R decided by *rpoB* sequencing OR • Heteroresistance / mixed population

Adapted from slide by N Beylis

Discordant Rifampicin Results (4)
XPERT-S : LPA-S : Pheno-DST-R

Scenario	Explanation
Uncommon as if Xpert-S, algorithm only allows 2nd specimen for microscopy, not LPA	• *rpoB* mutations outside the RRDR • Efflux pumps are not be detected by Xpert or LPA • Limit of detection for genotypic tests is above the level of the Rifampicin-R mutant in the sample

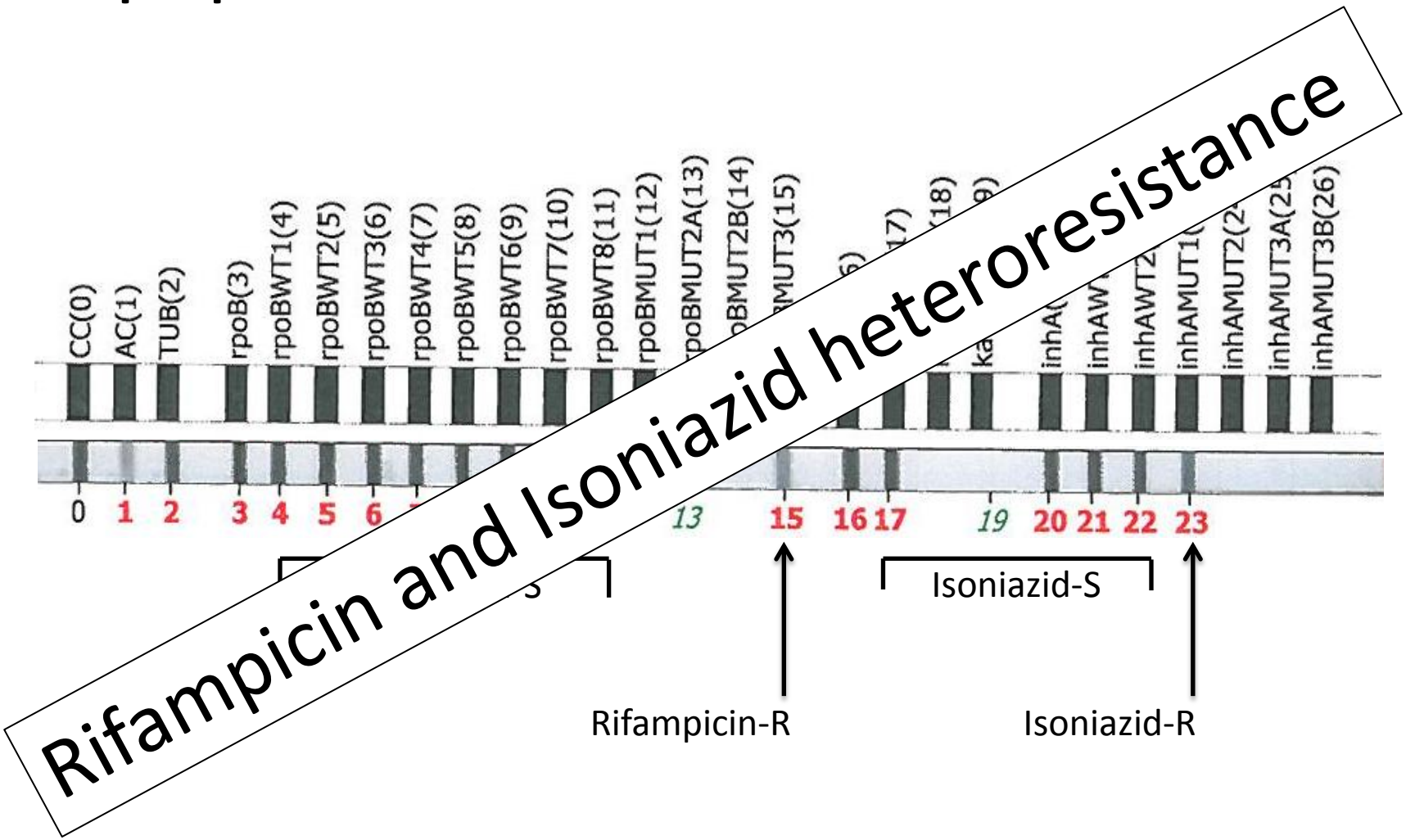
Adapted from slide by N Beylis

Discordant Isoniazid Results
LPA-S : Phenotypic-DST-R

- Isoniazid resistance
  - 60-70% due to *katG* mutation (high level)
  - 10-20% due to *inhA* mutation (low level)
- Resistance engendered by non-*katG*/*inhA* mutation mechanisms will not be picked up

Adapted from slide by N Beylis

What does heteroresistance or mixed populations look like on the LPA?



Due to the current diagnostic approach, which relies on Xpert as the starting point, heteroresistance usually becomes apparent either once the LPA is performed, if multiple samples get through the system, or the patient presents with ‘failure to thrive’

Clinical outcomes of patients with discordant diagnostic tests, heteroresistance or mixed infections

Mixed *Mycobacterium tuberculosis* Complex Infections and False-Negative Results for Rifampin Resistance by GeneXpert MTB/RIF Associated with Poor Clinical Outcomes

Nicola M. Zetola,[a,c,d] Sanghyuk S. Shin,[h] Kefentse A. Tumedi,[a] Keletso Moeti,[b] Ronald Ncube,[e] Mark N
Jeffrey D. Klausner,[h] Chawangwa Modongo[a,c]

- Retrospective cohort study in
- Data from the National rogram
- Predictors of po me
  - Xpert R -R

  OR .1-20.5) p<0.001

  cobacterium tuberculosis infections

  .5 (95% CI 1.2-48.2) p=0.03

Should Xpert RIF-S tuberculosis be interrogated further in high prevalent TB endemic setting?

JCM 2014;52(7):2422-9

Managing mixed infections

Treat for both DS-TB and DR-TB

How should we treat patients with disputed *rpoB* mutations?

Rifampin Drug Resistance Tests for Tuberculosis: Challenging the Gold Standard

Armand Van Deun,[a,b] Kya J. M. Aung,[c] Valentin Bola,[d] Rossin Lebeke,[d] Mohamed Anwar Hossain,[c] Willem Bram de Rijk,[a] Leen Rigouts,[a] Aysel Gumusboga,[a] Gabriela Torrea,[a] Bouke C. de Jong[a]

- Sequenced sputum from 1st failure or relapse
- 10.6% samples from Kinshasa and 13.1% from Bangladesh had disputed mutations
  - 511Pro, 516Tyr, 526Asn, 526Leu, 533Pro, 572Phe

Van Deun JCM 2013;51(8):2633-40

Rifampin Drug Resistance Tests for Tuberculosis: Challenging the Gold Standard

Armand Van Deun,[a,b] Kya J. M. Aung,[c] Valentin Bola,[d] Rossin Lebeke,[d] Mohamed Anwar Hossain,[c] Willem Bram de Rijk,[a] Leen Rigouts,[a] Aysel Gumusboga,[a] Gabriela Torrea,[a] Bouke C. de Jong[a]

- No difference in treatment failure (63%) with 1st line TB therapy between those with disputed vs undisputed *rpoB* mutations
- Other smaller studies by Williamson (NZ) and van Ingen (Netherlands) similar findings

Williamson. IJTLD 2012;16:216-20 Van Ingen. IJTLD 2011;15:990-2 Van Deun JCM 2013;51(8):2633-40

Conclusions

- Discordance between genotypic and phenotypic tests are increasingly recognized and often rely on genome sequencing to elucidate the mechanism
- High rates of *Mycobacterium tuberculosis* transmission in high endemicity populations increase the prevalence of mixed infections

Conclusions (2)

- Patients with mixed populations of *Mycobacterium tuberculosis* should be treated for both DS-TB and DR-TB
- Patients with disputed *rpoB* mutations should be treated for MDR-TB ± high dose rifampicin as clinical outcome is worse with standard treatment