# Drug resistant TB: The role of the laboratory




NATIONAL HEALTH
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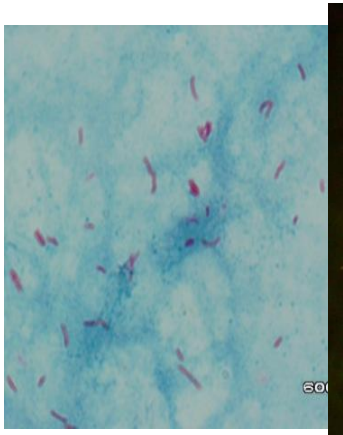
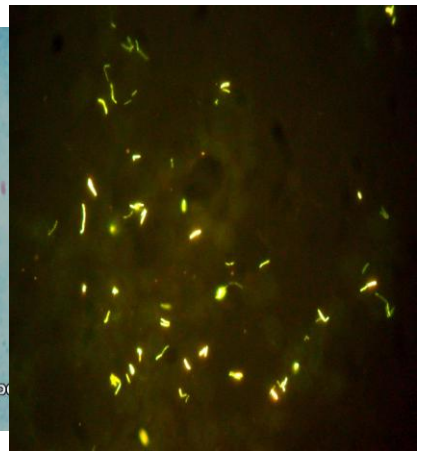
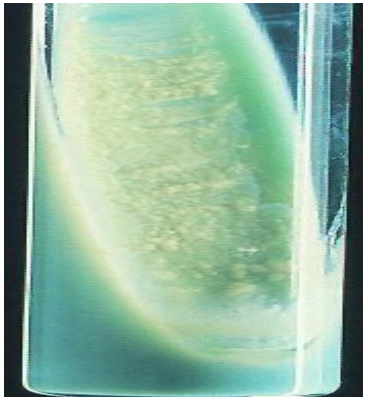
Andrew Whitelaw
NHSLS / UCT

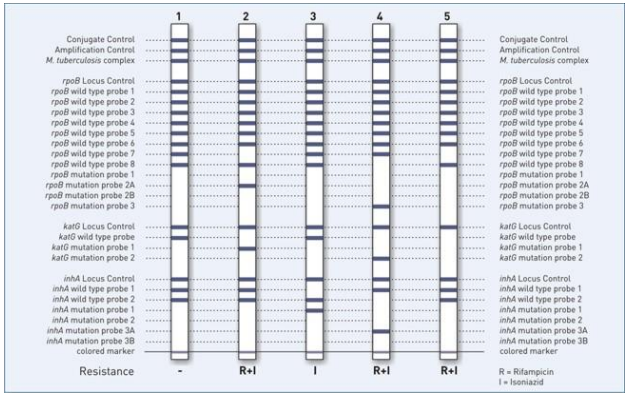
26 Oct 2012

# TB lab functions:










# Outline

- Resistance testing
  - Genotypic
  - Phenotypic
- Which tests are done when, and why…
- Reporting of results

- All in an effort to standardise across NHLS!

# Phenotypic

- Does the organism grow in the presence of the antibiotic?
  - Solid or liquid culture
  - Concentration of antibiotic
  - Purity of culture
  - Experience of technologist
- What is “gold standard”
  - Still debatable – probably agar proportion method
- What does NHLS use
  - MGIT or agar proportion (ideally want MGIT for all)

# Which antibiotics

- Theoretically any!

- Reproducibility
- Reliability
- Convenience / ease
- Correlation with clinical response

- Rif and INH well established, well accepted
- Aminoglycosides, quinolones also accepted
- Ethambutol, ethionamide, PZA, streptomycin - ?

# Three questions…

- What is reliable?
- What is useful?
- What is practical?

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- What is useful?
- What is practical?

TABLE 1. Test results for clinical strains of *M. tuberculosis* ($n$ = 200) for susceptibility to SIRE and PZA

| Antibiotic (concn [μg/ml]) | MGIT 960 result[a] | RRM result[a] | | Overall agreement (%) | Sensitivity[b] (%) | Specificity[c] (%) | PPV[d] (%) | NPV[e] (%) |
|---|---|---|---|---|---|---|---|---|
| | | S | R | | | | | |
| STR (1.0) | S | 37 | 6 | 97 | 96 | 100 | 100 | 86 |
| | R | | 157 | | | | | |
| STR (4.0) | S | 82 | 16 | 92 | 86 | 100 | 100 | 84 |
| | R | | 102 | | | | | |
| INH (0.1) | S | 30 | 3[f] | 99 | 98 | 100 | 100 | 91 |
| | R | | 167 | | | | | |
| INH (0.4) | S | 31 | 3[f] | 99 | 98 | 100 | 100 | 91 |
| | R | | 166 | | | | | |
| RIF (1.0) | S | 68 | | 100 | 100 | 100 | 100 | 100 |
| | R | | 132 | | | | | |
| EMB (5.0) | S | 103 | 71 | 65 | 27 | 100 | 100 | 59 |
| | R | | 26 | | | | | |
| EMB (7.5) | S | 156 | 33 | 84 | 25 | 100 | 100 | 83 |
| | R | | 11 | | | | | |
| PZA (100)[g] | S | 158 | 8 | 92 | 77 | 98 | 90 | 95 |
| | R | 3 | 26 | | | | | |

[a] S, susceptible; R, resistant. Values are numbers of isolates.
[b] The sensitivity, i.e., the ability of MGIT 960 to detect true resistance compared with the RRM results.
[c] The specificity, i.e., the ability of MGIT 960 to detect true susceptibility compared with the RRM results.
[d] PPV, positive predictive value.
[e] NPV, negative predictive value.
[f] Mixed cultures (consisting of resistant and susceptible subpopulations).
[g] Five *M. tuberculosis* isolates did not grow in one of the systems used.

Kruuner JCM 2006

200 clinical isolates, varying susc patterns
RRM – resistance ratio method (on LJ)

TABLE 3. Test results for clinical strains of MDR *M. tuberculosis* ($n$ = 133) for susceptibility to second-line drugs

| Antibiotic (concn [μg/ml]) | MGIT 960 result[a] | RRM/MPA[a,b] result | | Rate of agreement (%) | Sensitivity[c] (%) | Specificity[d] (%) | PPV[e] (%) | NPV[f] (%) |
|---|---|---|---|---|---|---|---|---|
| | | S | R | | | | | |
| AMI (1.0) | S | 108 | 1 | 99 | 96 | 99 | 96 | 99 |
| | R | | 23 | | | | | |
| CAP (1.25) | S | 105 | 2 | 97 | 92 | 98 | 92 | 98 |
| | R | 2 | 24 | | | | | |
| OFL (1.0)[b] | S | 97 | | 100 | 100 | 100 | 100 | 100 |
| | R | | 34 | | | | | |
| RIFB (0.5) | S | 6 | 2 | 99 | 98 | 100 | 100 | 75 |
| | R | | 125 | | | | | |
| PRO (2.5) | S | 119 | 3 | 96 | 75 | 98 | 82 | 98 |
| | R | 2 | 9 | | | | | |
| PRO (5.0) | S | 124 | 3 | 97 | 63 | 99 | 83 | 98 |
| | R | | 5 | | | | | |

[a] S, susceptible; R, resistant.
[b] Values are numbers of isolates. A total of 132 *M. tuberculosis* clinical isolates were tested.
[c] The sensitivity, i.e., the ability of MGIT 960 to detect true resistance compared with the RRM results.
[d] The specificity, i.e., the ability of MGIT 960 to detect true susceptibility compared with the RRM results.
[e] PPV, positive predictive value.
[f] NPV, negative predictive value.

Kruuner JCM 2006

133 MDR clinical isolates

**TABLE 3** Success rate of detecting drug resistance or susceptibility as determined by the majority result for each drug[a]

| Drug by method | No. of tests with expected susceptibility | Success rate (%) for susceptible specimens | No. of tests with expected resistance | Success rate (%) for resistant specimens |
|---|---|---|---|---|
| Pyrazinamide | | | | |
| Bactec | 5,843 | 96.8 | 1,013 | 98.1 |
| MGIT | 1,399 | 95.9 | 255 | 94.9 |
| Ethambutol | | | | |
| 7H10 agar | 3,050 | 98.4 | 326 | 78.5 |
| 7H11 agar | 365 | 98.1 | 40 | 52.5 |
| Bactec | 8,362 | 98.4 | 823 | 88.0 |
| MGIT | 1,794 | 97.6 | 203 | 48.3 |
| INH (total) | | | | |
| AP | 4,920 | 98.7 | 2,079 | 92.9 |
| Bactec | 6,796 | 98.7 | 3,438 | 91.6 |
| MGIT | 1,793 | 97.1 | 837 | 92.6 |
| INH low level | | | | |
| Agar | 2,203 | 99.0 | 610 | 82.8 |
| Bactec | 5,236 | 98.6 | 1,471 | 82.3 |
| MGIT | 1,293 | 97.0 | 275 | 79.6 |
| Rifampin | | | | |
| Agar | 3,171 | 99.7 | 728 | 94.4 |
| Bactec | 7,098 | 99.7 | 1,460 | 95.0 |
| MGIT | 1,652 | 99.2 | 288 | 66.7 |

[a] Resistance or susceptibility for each drug is defined as the majority result of >50% reported by all participants that used the CLSI reference method.

100-120 labs across US
Panels distributed annually
Majority agreement ref standard

2 strains with borderline rif resistance (His526Leu)

Angra, J Clin Micro, 2012

TABLE 1. Comparison of results obtained by use of MGIT 960 and AP[a]

| Drug | No. of strains with indicated results by AP/MGIT 960[b] | | | | Overall agreement[c] |
|---|---|---|---|---|---|
| | Agreement | | Discrepancy | | |
| | R/R | S/S | R/S | S/R | |
| LVX | 28 | 88 | 0 | 1[d] | 99.1 (116/117) |
| AMK | 37 | 80 | 0 | 0 | 100 (117/117) |
| CAP | 35 | 79 | 0 | 3[d] | 97.4 (114/117) |
| ETH | 43 | 61 | 2[e] | 11[f] | 88.9 (104/117)[g] |

[a] A total of 117 strains were tested.

[b] R, resistant; S, susceptible.

[c] The data represent percent agreement (number of strains with the correct result/total number of strains tested).

[d] Tested resistant with the Bactec 460 system.

[e] One of two strains tested susceptible with the Bactec 460 system.

[f] Ten of 11 strains tested resistant with the Bactec 460 system.

[g] The results obtained with the MGIT 960 and Bactec 460 systems were concordant for 11 of 13 discrepancies.

Grace Lin, JCM, 2009

# What about PZA

- Used to be problematic
- New MGIT kit – good results

- Concern with false resistance (14/57  Chedore et al)

# Genotypic

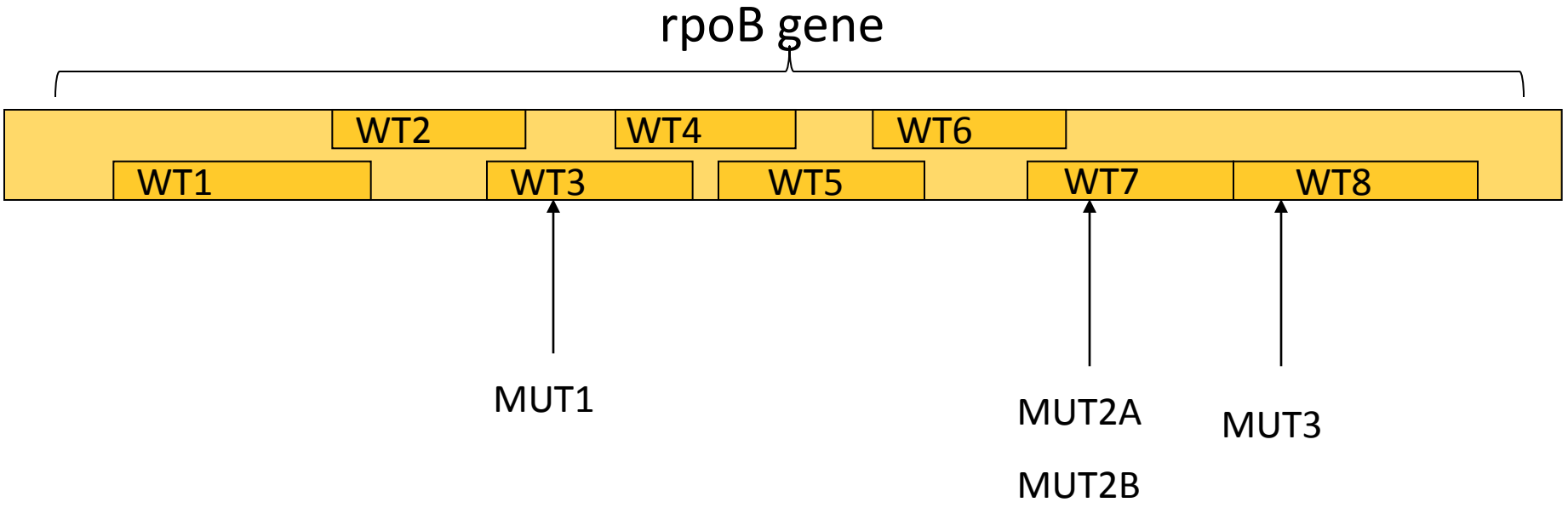
- Look for mutations that confer resistance

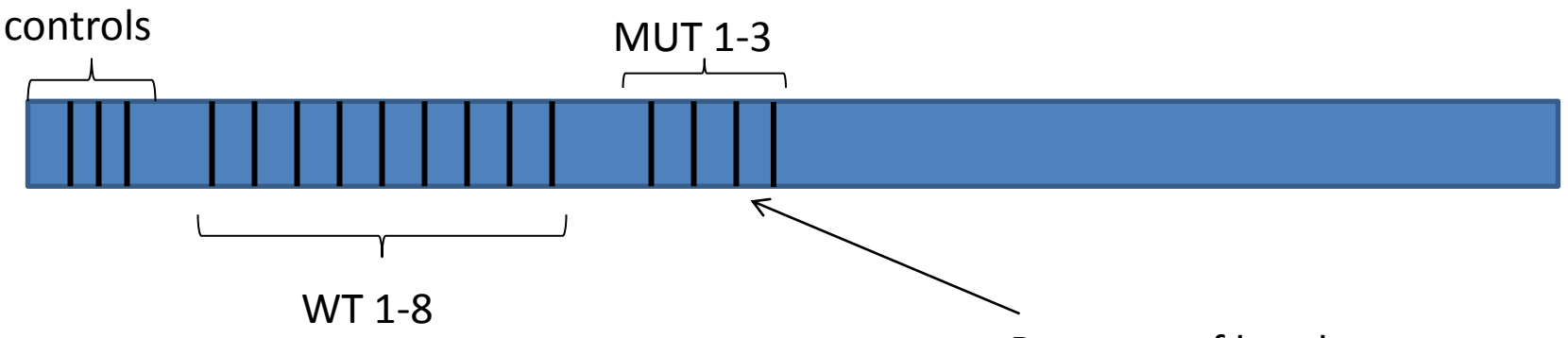
- Only works if mutations known!
- Quicker
- Potentially more difficult
- 3 in widespread use
  - GeneXpert MTB/Rif (Cepheid)
  - MTBDRplus (Hain Life Sciences)
  - MTBDRsl (Hain Life Sciences)

# Line probe assays

- Hain LifeSciences MTBDRplus kit
  - Detection of rifampicin and isoniazid resistance in M. tb
  - >95% rif resistance due to mutations in a specific region of rpoB gene
  - 70-80% INH resistance due to mutations in either katG or inhA
- Hain LifeSciences MTBDRsl kit
  - Resistance to fluoroquinolones, injectable agents, ethambutol
  - Works fairly well for FQs, injectables; poor for ethambutol

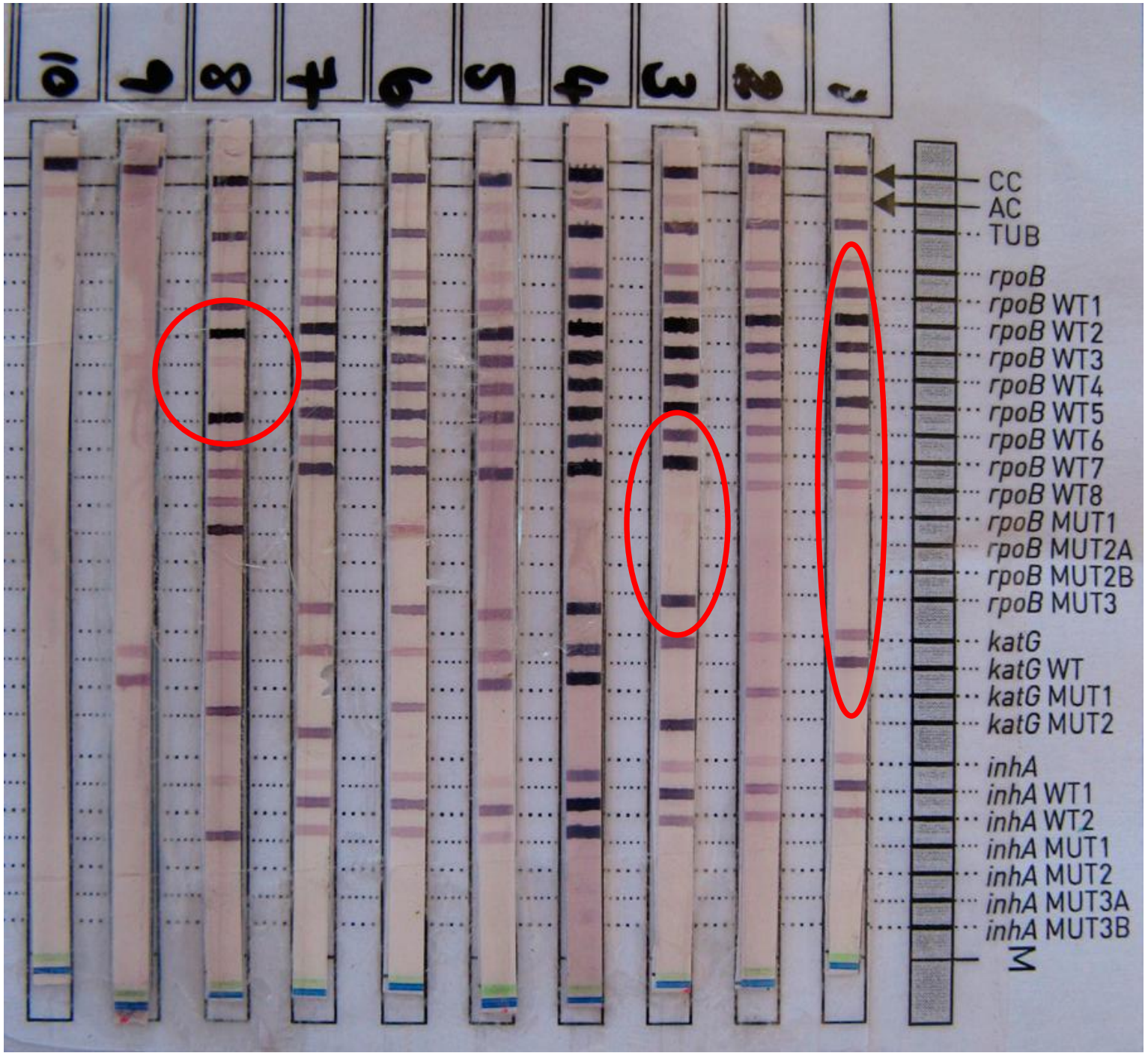
# Line probe assay - MTBDRplus




Presence of band = known resistance mutation

Absence of band (with no MUT band) = mutation in rpoB


CC
AC
TUB
rpoB
rpoB WT1
rpoB WT2
rpoB WT3
rpoB WT4
rpoB WT5
rpoB WT6
rpoB WT7
rpoB WT8
rpoB MUT1
rpoB MUT2A
rpoB MUT2B
rpoB MUT3
katG
katG WT
katG MUT1
katG MUT2
inhA
inhA WT1
inhA WT2
inhA MUT1
inhA MUT2
inhA MUT3A
inhA MUT3B
M

# rpoB

- Rif resistance – detects >98%
- Missing WT plus MUT = resistance
- Missing WT alone = ?

- Up to 50% of missing WT/absent MUT susceptible (varies acc to which band)
- Also possible geographic variation
- Limitation of susc testing methodology
  - MICs being planned
  - Clinical relevance?

Beylis, unpublished

# inhA and katG

- Overall LPA detects 70-90% INH resistance
- katG mutation
  - Predicts high level INH resistance (80-92%)
  - No prediction of ETH
- inhA mutation
  - Predicts low level INH resistance (78-90%)
  - Predicts ETH resistance (50-80%)
  - WT inhA does not = ETH susceptible…

Barnard, AJRCCM, 2008
Kim, DMID, 2003
Brossier, JCM, 2006
Morlock, AAC, 2003
Schaaf, IJTLD, 2009

# Real time PCR

- GeneXpert
  - Amplify portion of rpoB gene most commonly linked to mutations (RRDR – rifampicin resistance determining region)
  - 5 Molecular beacons designed to overlap and cover entire 81bp region

Concentrates bacilli & removes inhibitors

**Sample is automatically filtered & washed**

4

**Ultrasonic lysis of filter-captured organisms to release DNA**

5

**DNA molecules are mixed with dry PCR reagents**

6

**Semi-nested real-time amplification & detection in integrated reaction tube**

7


**Time-to-result: 1 h 45 min**

Printable test result

1

**Sputum liquefaction & inactivation with 2:1 SR**

2

**Transfer of 2 ml after 15 min**

End of hands on work

3

GeneXpert® Dx System

User | Data Management | Trending | Setup | Advanced Setup | View Results | About | User Ana Milovic

Create Test | Check Status | Stop Test | View Results | Define Assays | Define Graphs | Maintenance

Module Name B2
Sample ID* X21802843
Assay MTB Beta
Assay Version 6
Assay Type Research Use Only
Reagent Lot ID 00601
Cartridge S/N 0
Expiration Date <None>
Test Type Specimen

Notes

Start Time 10/9/2008 12:52:00
End Time 10/9/2008 14:22:55
Status Done
Error Status OK
User Ana Milovic
S/W Version 2.1
Instrument/Module S/N 703771/602299

Views
Result View
Primary Curve

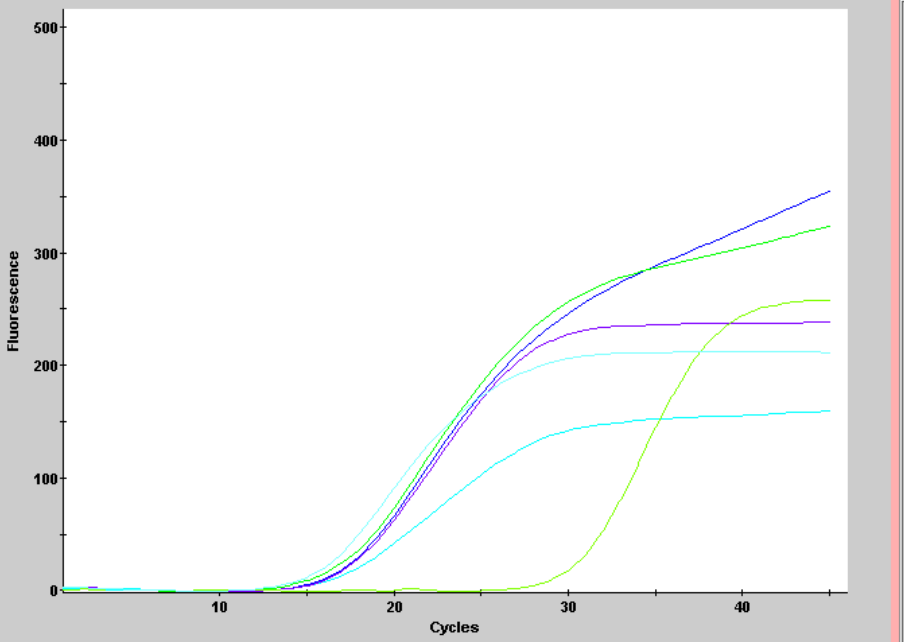
Test and Analyte Result | Detail | Errors | History

Assay Name MTB Beta  Version 6

Test Result MTB POSITIVE HIGH;
Rif Resistance NOT DETECTED

| Analyte Name | Ct | EndPt | Analyte Result | Probe Check Result |
|---|---|---|---|---|
| Probe D | 17.1 | 355.0 | POS | PASS |
| Probe C | 16.5 | 323.0 | POS | PASS |
| Probe E | 17.9 | 159.0 | POS | PASS |
| Probe B | 17.3 | 238.0 | POS | PASS |
| Bg | 30.2 | 258.0 | NA | PASS |
| Probe A | 16.0 | 211.0 | POS | PASS |

Views
Result View
Primary Curve




Legend
Probe D; Primary
Probe C; Primary
Probe E; Primary
Probe B; Primary
Bg; Primary
Probe A; Primary

Save Changes | Export | Report | Select Graphs | View Test

start | GeneXpert® Dx System | Document1 - Microsof... | 12:10 PM

GeneXpert® Dx System

User Data Management Trending Setup Advanced Setup View Results About User Ana Milovic

Create Test | Check Status | Stop Test | View Results | Define Assays | Define Graphs | Maintenance

Module Name B2
Sample ID* X21802632
Assay MTB Beta
Assay Version 6
Assay Type Research Use Only
Reagent Lot ID 00601
Cartridge S/N 0
Expiration Date <None>
Test Type Specimen

Notes

Start Time 10/3/2008 15:22:05
End Time 10/3/2008 16:52:47
Status Done
Error Status OK
User Ana Milovic
S/W Version 2.1
Instrument/Module S/N 703771/602299

Views
Result View
Primary Curve

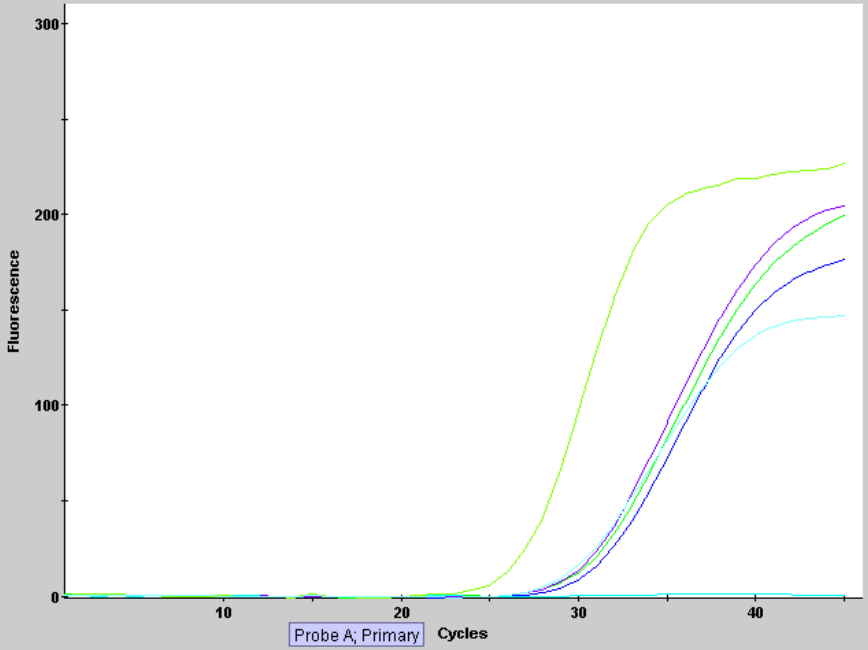
Test and Analyte Result | Detail | Errors | History

Assay Name MTB Beta  Version 6

Test Result MTB POSITIVE VERY LOW; Rif Resistance DETECTED

| Analyte Name | Ct | EndPt | Analyte Result | Probe Check Result |
|---|---|---|---|---|
| Probe D | 31.4 | 177.0 | POS | PASS |
| Probe C | 30.9 | 200.0 | POS | PASS |
| Probe E | 0.0 | 1.0 | NEG | PASS |
| Probe B | 30.7 | 204.0 | POS | PASS |
| Bg | 26.6 | 227.0 | NA | PASS |
| Probe A | 30.4 | 147.0 | POS | PASS |

Views
Result View
Primary Curve




Legend
Probe D; Primary
Probe C; Primary
Probe E; Primary
Probe B; Primary
Bg; Primary
Probe A; Primary

Save Changes | Export | Report | Select Graphs | View Test

start | GeneXpert® Dx System | Document1 - Microsof... | 12:19 PM

# How well does it work – for rif?

| | Concordant LPA (RIF) | Discordant LPA (RIF) | Concordant Culture (RIF) | Discordant Culture (RIF) |
|---|---|---|---|---|
| Eastern Cape | 22 | 1 | 1 | 31 |
| Free State | 21 | 3 | 3 | 2 |
| KZN | 218 | 27 | 219 | 13 |
| Limpopo | 12 | 2 | 20 | 1 |
| Mpumalanga | 65 | 10 | 55 | 0 |
| North West | 12 | 1 | 2 | 1 |
| Gauteng | 48 | 5 | 15 | 3 |
| Northern Cape | 49 | 12 | 10 | 5 |
| Western Cape | 142 | 7 | 2 | 0 |
| | **589** | **68** | **327** | **56** |
| Concordance (%) | 89.6% | | 85.4% | |

http://www.stoptb.org/wg/gli/assets/html/4th%20GLI%20meeting%2017-19%20April%202012%20AGENDA.htm

85-90% of rif resistant results on Xpert are “true resistance”

# Recap

**Phenotypic**

- Rif, INH, amik, kana, oflox, moxiflox all reproducible
- PZA – some concerns
- Ethionamide – MGIT may be better (?)
- Ethambutol – not as reliable

**Genotypic**

- LPA
  - Great for Rif
  - 70-90% sens for INH, good specificity
  - katG vs inhA givs some extra info
- Xpert
  - Rif sens – reliable
  - Rif resistant needs confirmation

# Three questions…

- What is reliable?
- What is useful?
- What is practical?

# GeneXpert Diagnostic Algorithm

**TB Suspects**
(New, Retreatment, Prisoners, Health Care Workers, Contacts of confirmed drug sensitive and drug resistant TB patients)

Collect **TWO** spot sputum specimens **ONE** hour apart
Complete sputum request forms and indicate HIV status of patient in remarks section on the sputum request form.
Staple the two plastic bags together, each plastic bag containing one sputum specimen, for dispatch to NHLS.

Clinic

| GX Positive Rif Susceptible | GX Positive Rif Resistant | GX Positive Rif Inconclusive | GX Negative | GX Unsuccessful |
|---|---|---|---|---|
| NHLS

NHLS to use 2nd sample to do microscopy | NHLS use 2nd sample to do microscopy, culture & DST | NHLS use 2nd sample to do microscopy, culture & DST | If patient HIV+ve, NHLS will use 2nd specimen to do cul & DST, if HIV-ve NHLS will discard 2nd specimen | NHLS use 2nd sample to repeat GX |
| Clinic

Start TB Treatment | Recall patient for review by PHC MO | Start DS TB Treatment | HIV +ve / HIV -ve | If 2nd GX unsuccessful send 3rd specimen for microscopy, culture & DST |
| Smear +ve / Smear -ve | Patients well, wait for cul & DST / Patient unwell, start DR & DS Rx | Cul +ve & DST resistant stop DS Rx & start DR Rx / Cul +ve & DST sensitive continue DS Rx | Rx with anti-biotics, Request chest X-ray. Make appointment to see MO / Rx with antibiotics | |
| Record as Smear +ve / Record a Smear -ve | Cul +ve & DST resistant start DR Rx / Cult +ve & DST resistant stop DS Rx | | MO to decide to start Rx or wait for results, if cul +ve & DST sensitive start TB Rx / Review in 5 days | |
| Do micros-copy at conversion & discharge / Do culture at conversion | | | Well, discharge or if unwell, refer | |

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Provincial Government of the Western Cape

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# DST as it stands

- Xpert Rif susceptible
  - No further DST
  - INH mono-resistance will be missed
- Xpert Rif resistant
  - Confirm Rif (LPA and/or phenotypic)
  - 2nd line DST on isolate
- Xpert negative / culture pos
  - LPA Rif and INH
  - LPA Rif resistant – 2nd line DST

**Table XIII Suggested Regimens for Mono- and Poly-Drug Resistance in Patients where Further Acquired Resistance is not a Factor**

| Drug resistance pattern | Suggested regimen | Minimum duration of treatment (months) | Comments |
|---|---|---|---|
| H | Continue Regimen I or II intensive phase for full duration of treatment (except for H).<br><br>In practice it is easier fixed drug combinations RHZ + EMB for children < 8 years and RHZE for individuals > 8 years. | 6 - 9 months based on symptomatic response to treatment, weight gain and sputum culture combinations.<br><br>A minimum of 6 months treatment after culture conversion is adequate. | Monitor the patient with sputum smear microscopy and culture on monthly basis throughout treatment.<br><br>Monthly clinical assessment required.<br><br>Refer to MDR-TB expert at unit if patient is not responding well to treatment. |
| R<br><br>(+/- any other 1st line drug than H) | Standardised MDR-TB regimen plus INH. | 18 months after culture conversion | These patients will need confirmation of diagnosis if diagnosed through GXP; however, LPA is a confirmatory diagnosis. |
| Poly-resistant TB cases | | | Refer to MDR-TB expert for regimen design based on resistance pattern and history of anti-tuberculosis drugs used. |

Management of Drug Resistant Tuberculosis Policy Guidelines 2011

# INH mono-resistance?

- Guidelines suggest standard therapy

- In practice?
  - Add moxiflox
  - Add ethionamide
  - Add other?
- Outcomes of INH mono-resistant TB?

# MDR treatment

- Kanamycin / amikacin
- Moxifloxacin (levofloxacin in children)
- PZA
- Terizidone
- Ethionamide

- Adjust once DST available

- “Ethambutol may be used as an additional item (sixth item in the standardised regimen) in areas with confirmed low prevalence to ethambutol resistance or in patients who have not received ethambutol for more than one month before DR-TB treatment.”

Management of Drug Resistant Tuberculosis Policy Guidelines 2011

# XDR treatment

- Capreomycin
- Moxifloxacin (levofloxacin)
- Ethionamide
- Terizidone
- Pyrazinamide
- PAS
- Clofazimine

# Three questions…

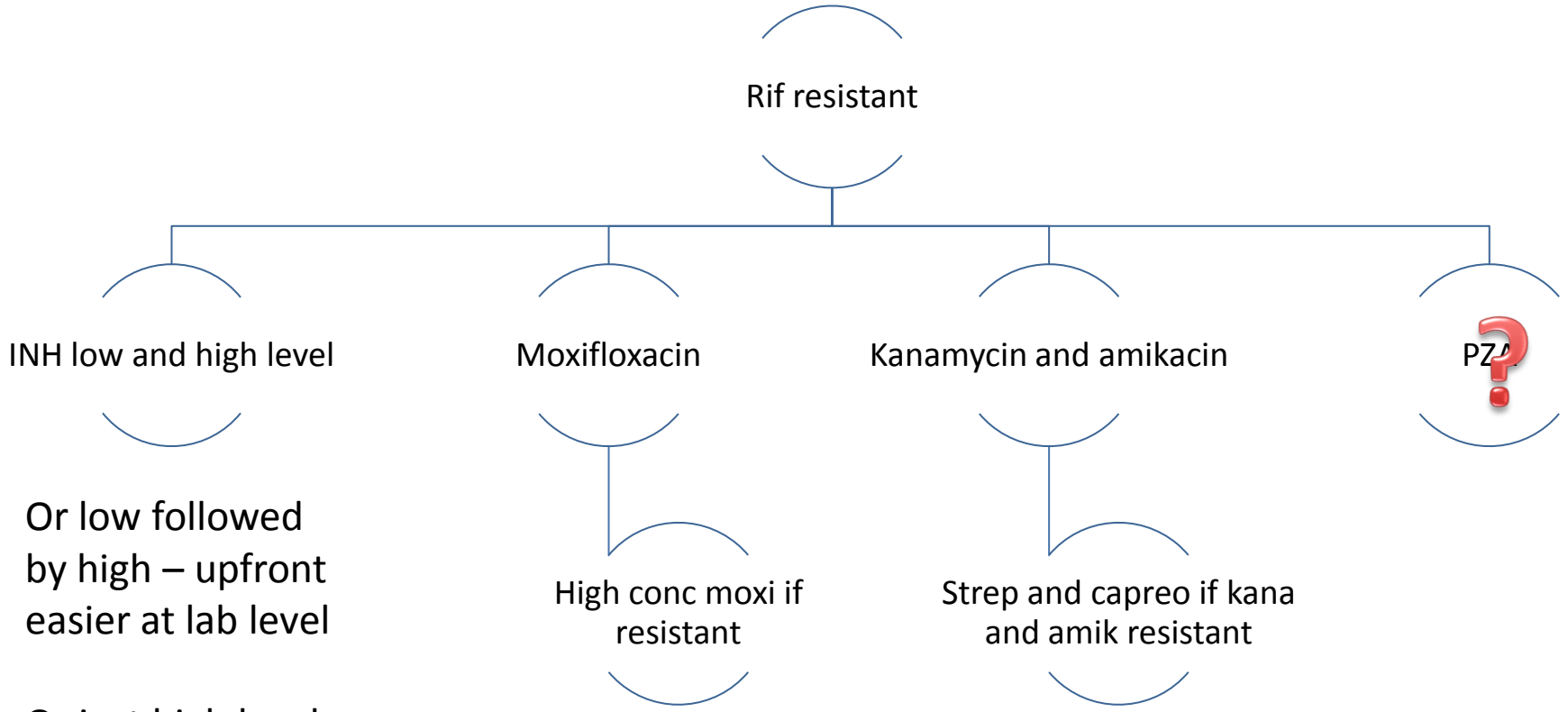
- What is reliable?
- What is useful?
- What is practical?

# Going forward

- Standardise methodology
  - LPA first line
  - MGIT for phenotypic
    - Expensive but quicker
    - Will be challenging to roll out…
- Centralise SLD – up to a point
  - No formal plan as yet
  - Logistics must improve


South Africa
Zimbabwe
Messina
Ellisras
Limpopo Province
Thabazimbi
Kruger National Park
Mocambique
Botswana
Rustenburg
Witbank
North-West
Nelspruit
Mpumalanga
Swaziland
Vryburg
Harrismith
Namibia
Free State
KwazuluNatal
Upington
Springbok
Kimberley
Bloemfontein
Pietermaritzburg
Lesotho
Durban
Northern Cape
Atlantic Ocean
Clanwilliam
Eastern Cape
Indian Ocean
Beaufort West
East London
Western Cape
Cape Town
George
Port Elizabeth

# Standardise SLD




Or low followed by high – upfront easier at lab level

Or just high level – if "S", add INH

Levofloxacin?
Moxi R seems to predict levo R

# INH mono-resistant

- Relex testing for
  - Moxiflox
  - ?PZA
  - ?Ethionamide

- BUT – many (most) INH mono-R cases will be missed in any case…

# Standardise reporting

- LPA –Rif
  - WT missing, no MUT band = inconclusive
  - Confirm with phenotypic (ideally MICs if possible)
- LPA – INH
  - Report on katG vs inhA?
  - Wording of comment challenging
- LPA – mixed
  - Treat as MDR but add Rif / INH

# MTBDRsl?

- Fine for FQs, injectables
- Poor for EMB

- Potentially offer MTBDRsl, but needs confirmation with phenotypic DST
- Will it cause confusion at clinic level?

# Unresolved issues

- Ethionamide
  - ?on request
  - ?routine
- Ethambutol
  - On request only, ? Useful
- MTBDRsl
  - Valuable, but maybe confusing as well

# Acknowledgements


- TB standardisation committee
- Wendy Stevens
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- Hendrik Koornhof
- Marlein Bosman
- Marinus Barnard
- John Simpson