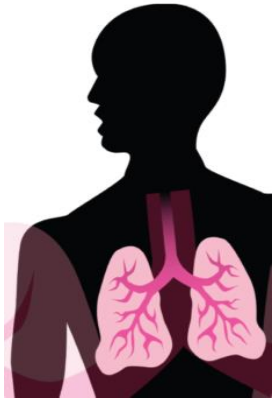


Molecular diagnostics: implications for future NICE guidance

Onn Min Kon

NIHR HPRU in Respiratory Infections

NHS National Institute for Health Research

Imperial College London

Public Health England

MDR-TB Clinical Advice Service

British Thoracic Society

Imperial College Healthcare NHS Trust

A Clinical Perspective

- Rapid Molecular Tools
  - Rapid molecular tools versus smear versus culture
  - MDR TB versus ‘TB’ use
  - Extrapulmonary TB
- Whole Genome Sequencing

NICE 2016

Diagnosing pulmonary (including laryngeal) TB in adults

Request rapid diagnostic NAAT for the MTB complex on primary specimens if there is clinical suspicion of TB disease, ***and***:

- the person has HIV or
- rapid information about mycobacterial species **would alter the person's care** or
- the need for a large contact-tracing initiative is being explored. [new 2016]

Diagnosing pulmonary (including laryngeal) TB in children and young people

- In children aged ≤ 15 years with suspected pulmonary TB, offer rapid diagnostic NAAT for the MTB complex.

Usually only 1 NAAT is needed per specimen type (e.g. spontaneous sputum, induced sputum or gastric lavage). [new 2016]

- In young people aged 16–18 years use the same criteria as in adults. [new 2016]

NICE 2016 -extrapulmonary

- ‘Additional test if it would alter management’
  - Nodes/ intrathoracic
  - Pericardial
  - CSF

MDR TB

1.4.1 Multidrug-resistant TB

1.4.1.1 For people with clinically suspected TB, a TB specialist should request rapid diagnostic nucleic acid amplification tests for rifampicin resistance on primary specimens if a risk assessment for multidrug resistance identifies any of the following risk factors:

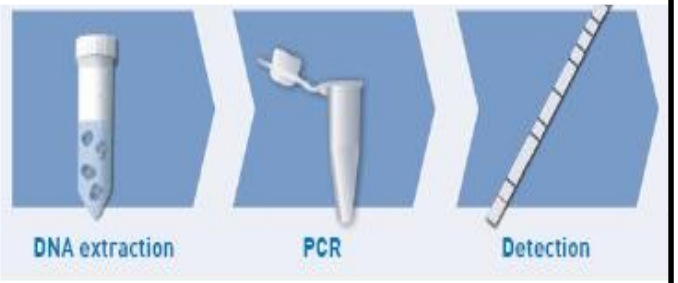
- history of previous TB drug treatment, particularly if there was known to be poor adherence to that treatment
- contact with a known case of multidrug-resistant TB
- birth or residence in a country in which the World Health Organization reports that a high proportion (5% or more) of new TB cases are multidrug-resistant.

Start infection control measures (see section 1.5). **[new 2016]**

Molecular Detection of MTB and drug resistance

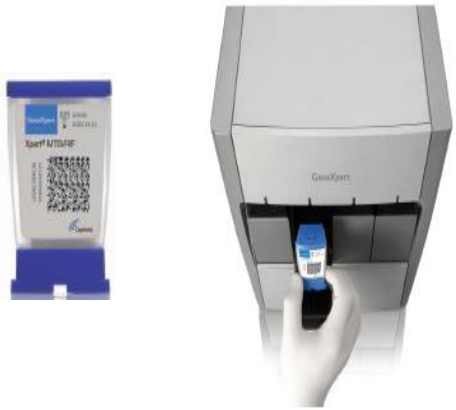
Molecular techniques used for DST is a rapid way of identifying resistance genes within organism, which aids diagnosis and implementation of treatment plans.

Commercial molecular tests for TB/MDR TB detection endorsed by WHO



GenoType MTBDR*plus*, MTBDR*sl, Nipro*

- Reverse hybridization, colorimetric reaction
- Results in 6-7 h
- some flexibility (n probes/strip: 30-40)
- Technical expertise: some
- Biosafety level 2



Xpert® MTB/RIF

- Integrated/automated qPCR
- Results in 2,5h
- Closed system (limited number of probes: <10)
- Technical expertise: none

Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults

Cochrane Database of Systematic Reviews 2013, Issue 1. Art. No.: CD009593.
Steingart KR, Sohn H, Schiller I, Kloda LA, Boehme CC, Pai M, Dendukuri N.

- Initial test replacing smear microscopy (15 studies, 7517 participants)
  - pooled sensitivity of 88%
  - pooled specificity of 98%
- Add-on test following a negative smear (14 studies, 5719 participants)
  - pooled sensitivity of 67%
  - pooled specificity of 98%
- **Smear +ve, culture +ve TB - pooled sensitivity was 98% (95%CrI 97% to 99%)**
- **Smear -ve, culture +ve TB - pooled sensitivity was 68% (95% CrI 59% to 75%)**
- HIV +ve - pooled sensitivity 80% (95% CrI 67% to 88%)
- Non HIV – pooled sensitivity 89% (95% CrI 81% to 94%)
- Rifampicin resistance detection (11 studies, 2340 participants)
  - pooled sensitivity of 94% (95% CrI 87% to 97%)
  - pooled specificity of 98% (95% CrI 97% to 99%)
- Distinguish between TB and nontuberculous mycobacteria (NTM)
  - 139 specimens with NTM, was positive in only 1 specimen

Xpert® MTB/RIF Versus AFB Smear and Culture to Identify Pulmonary Tuberculosis in Low Prevalence Settings

- US/Brazil/ South Africa
- 2 sputum samples in culture proven PTB
- Single sample
  - Xpert sensitivity in smear positive 85.2%
  - Xpert sensitivity in smear negative 59.3%
- Two samples
  - Xpert sensitivity in smear positive 100%*
  - Xpert sensitivity in smear negative 71.4%
- NPV in US:
  - Single NPV 99.7%
  - Two NPV 100%

* Deisolation potential

Luetkemeyer AF et al. Clin Infect Dis 2016;62(9):1081–8

Xpert® MTB/RIF Ultra*

- Detects Mtb by targeting two different multi-copy genes (*IS6110* & *IS1081*)
- Faster Time to Result
- PCR cycling optimized to improve sensitivity
- Melting temperature based analysis to improve RIF resistance detection

Xpert® MTB/RIF Ultra*

- 8 countries
  - South Africa, Uganda, Kenya, India, China, Georgia, Belarus, and Brazil
- 2368 participants for sputum sampling
  - 248 participants excluded
  - case detection group (n=1439)
  - multidrug-resistance risk group (n=314)
- ***smear-negative and culture-positive sputum***
  - **Xpert MTB/RIF Ultra 63% and Xpert MTB/RIF 46% (difference of 17%, 95% CI 10 to 24)**
- HIV-positive participants (115) with culture-positive sputum
  - Xpert MTB/RIF Ultra 90% and Xpert MTB/RIF 77% (13%, 6·4 to 21);
- ***all participants (462) with culture-positive sputum***
  - **Xpert MTB/RIF Ultra 88% and Xpert MTB/RIF 83% (5·4%, 3·3 to 8·0)**
- Xpert MTB/RIF Ultra and Xpert similar in detecting rifampicin resistance
- Specificities

  Xpert MTB/RIF Ultra 93% and Xpert 98% for patients *with a history of TB*

Dormann et al. Lancet Infect Dis 2018; 18: 76–84

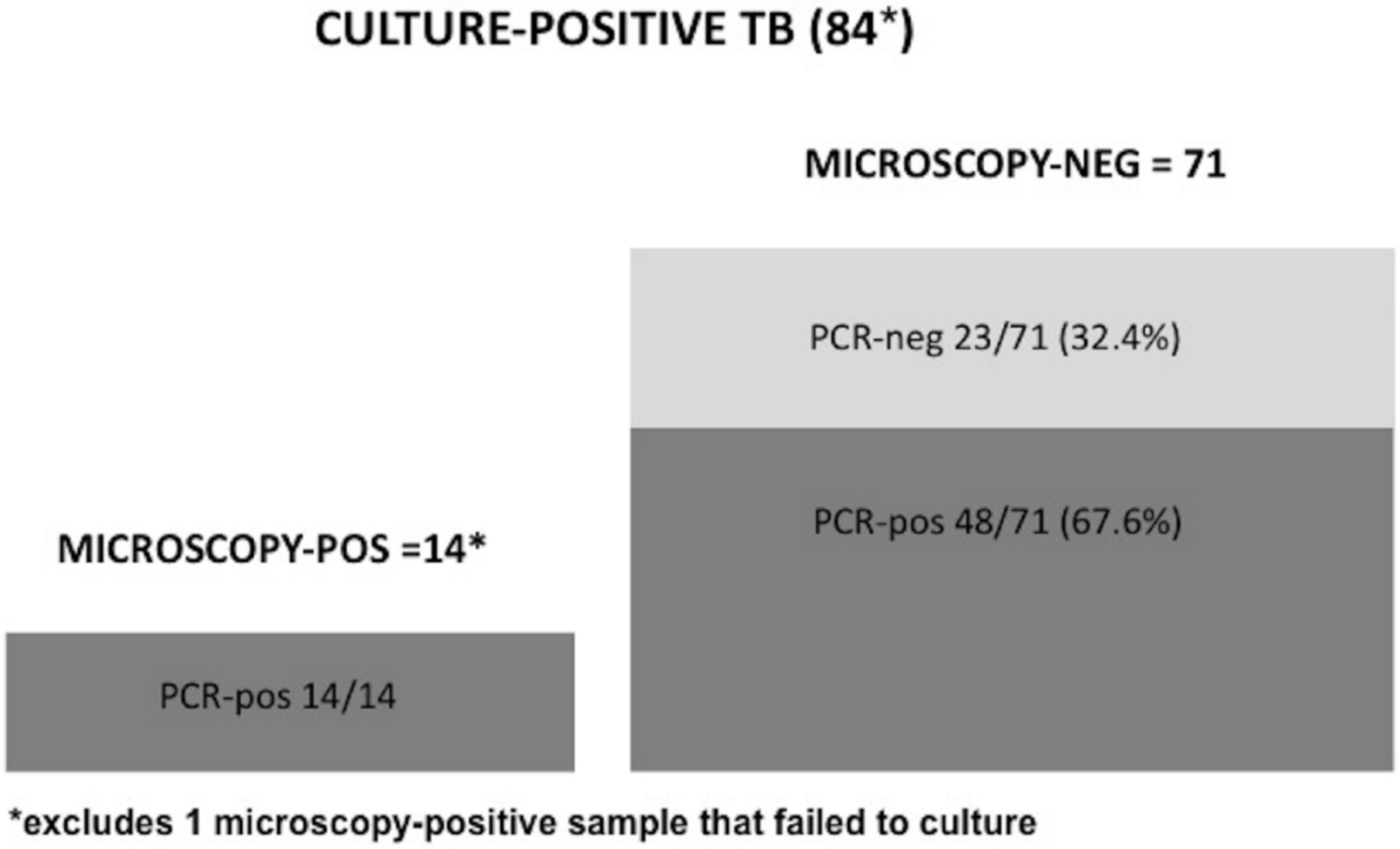


Figure 1. Breakdown of culture-positive transbronchial nodal aspirates (TBNA) – Microscopy/Culture vs. GeneXpert

Dhasmana D et al Ann Am Thor Soc March 2014

Table 2a. GeneXpert® and Cytology sensitivity data from both cohorts

	n	SENSITIVITY*
GENEXPERT®	84	72.6% (62.3-81.0)
CYTOLOGY	84	92.9% (85.3-96.7)
CYTOLOGY OR XPERT®	88	96.6% (90.5-98.8)

*CYTOLOGY' refers to TBNA grade 1-3; *95% confidence intervals in brackets*

- 9 cases PCR positive / culture negative
- 6 deemed to have active TB clinically

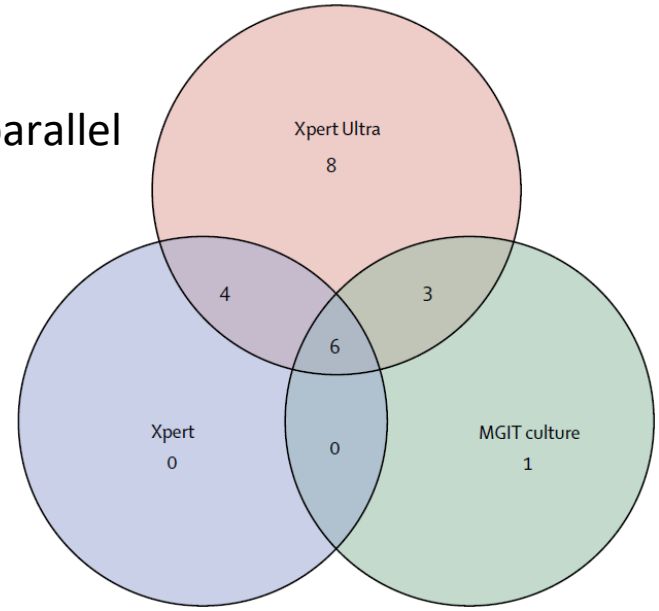
Dhasmana D et al Ann Am Thor Soc March 2014

First clinical evaluation of Xpert® MTB/RIF Ultra* in CSF^

Bahr et al. Lancet 2017

Study set up

- 128 HIV infected adults (Uganda)
- Culture, Xpert MTB/RIF and Xpert MTB/RIF Ultra in parallel
- Cerebrospinal fluid samples
- 107 samples TB negative in culture



First Clinical data on CSF Samples

- Sensitivity of Xpert MTB/RIF Ultra: **95%**
- Sensitivity of Xpert MTB/RIF: **43%**
- Sensitivity of MGIT culture: **43%**

Xpert MTB/RIF Ultra is **more sensitive** than Xpert MTB/RIF and culture in CSF samples

Source: Bahr N, et al, Diagnostic accuracy of Xpert MTB/RIF Ultra for tuberculous meningitis in HIV-infected adults: a prospective cohort study. Lancet Infect Dis. 2017 Sep 14. pii: S1473-3099(17)30474-7.

^ *Cerebrospinal fluid . Cepheid does not endorse the testing of alternate specimen types. If you choose to use Xpert MTB/RIF Ultra with alternate specimen types, it is your laboratory's responsibility to validate the assay for each alternate specimen type in accordance with federal, state, and local laws*

* CE-IVD. In Vitro Diagnostic Medical Device. Product distribution outside the United States. Product may not be available in all countries.

London MDR TB Cohort review data 2000-2016

- Most common countries of birth were India, UK, Somalia and Pakistan (accounting for 50% of patients)
- 79% had no previous history of TB
- 82% had no reported social risk factors
- 44% female

Country of birth	n
India	72
United Kingdom	34
Somalia	26
Pakistan	18
Lithuania	16
Romania	15
Unknown	12
Nepal	9
China	8
Nigeria	8
Afghanistan	7
Ukraine	7
Eritrea	5

Countries of birth with <5 cases are omitted

Courtesy of Charlotte Anderson/ Oliver McManus
Public Health England Health Protection Directorate: Field Epidemiology Service

London MDR TB Cohort review data 2000-2016

- All smear-positive MDR cases
  - 58% (93/160)
  - no prior treatment AND low-incidence MDR (<5%) countries
- All MDR cases regardless of smear result
  - 63% (259/412)
  - no prior treatment AND low-incidence MDR (<5%) countries

Courtesy of Charlotte Anderson/ Oliver McManus
Public Health England Health Protection Directorate: Field Epidemiology Service

Smear positive disease

- Confirms MTB complex
- More sensitive than smear
- Rapid diagnosis for MDR cases
  - MDR risks:
    - HIV
    - Social risk factors population
    - Prior treatment
    - High MDR prevalent epidemiologically

  OR
  - Significant insensitivity of current NICE criteria for MDR - hence case for testing all smear positives
  - (risk false positives)

Smear negative disease

- Confirms MTB pre-invasive sampling where access difficult or time critical
- Rapid diagnosis for potential MDR cases
  - Invasive sampling may be difficult
    - E.g. Central London social risk factors population – homelessness/ drug use etc
  - Prior treatment
  - High MDR prevalent epidemiologically

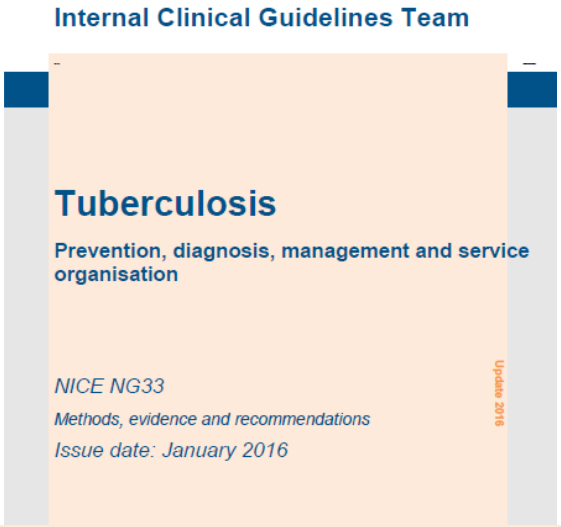
High Value Samples

In a **mixed pre-test probability** setting

- Bronchoalveolar lavage
- Mediastinal node aspirates
- CSF
- Node aspirates

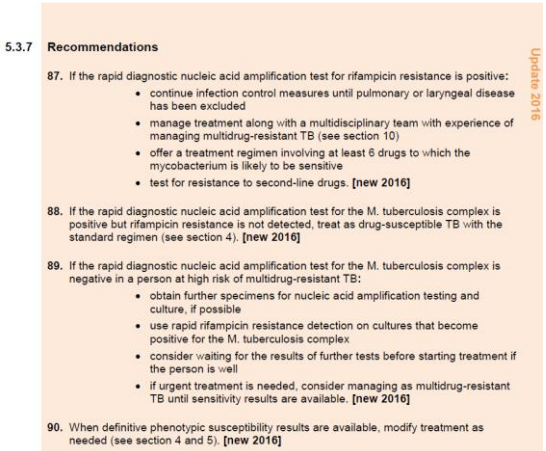
Diagnostic Use Locally

- All smear positive samples
- High value respiratory samples
- High risk MDR TB cases
- Extrapulmonary TB
- (Deisolation)



Adults

31. Request rapid diagnostic nucleic acid amplification tests for the *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. africanum*) on primary specimens (listed in 'Diagnostic investigations for pulmonary TB' table) if there is clinical suspicion of TB disease, and:
    - the person has HIV **or**
    - rapid information about mycobacterial species would alter the person's care **or**
    - the need for a large contact-tracing initiative is being explored. **[new 2016]**



5.3.7 **Recommendations**

Update 2016

87. If the rapid diagnostic nucleic acid amplification test for rifampicin resistance is positive:
    - continue infection control measures until pulmonary or laryngeal disease has been excluded
    - manage treatment along with a multidisciplinary team with experience of managing multidrug-resistant TB (see section 10)
    - offer a treatment regimen involving at least 6 drugs to which the mycobacterium is likely to be sensitive
    - test for resistance to second-line drugs. **[new 2016]**

88. If the rapid diagnostic nucleic acid amplification test for the M. tuberculosis complex is positive but rifampicin resistance is not detected, treat as drug-susceptible TB with the standard regimen (see section 4). **[new 2016]**

89. If the rapid diagnostic nucleic acid amplification test for the M. tuberculosis complex is negative in a person at high risk of multidrug-resistant TB:
    - obtain further specimens for nucleic acid amplification testing and culture, if possible
    - use rapid rifampicin resistance detection on cultures that become positive for the M. tuberculosis complex
    - consider waiting for the results of further tests before starting treatment if the person is well
    - if urgent treatment is needed, consider managing as multidrug-resistant TB until sensitivity results are available. **[new 2016]**

90. When definitive phenotypic susceptibility results are available, modify treatment as needed (see section 4 and 5). **[new 2016]**

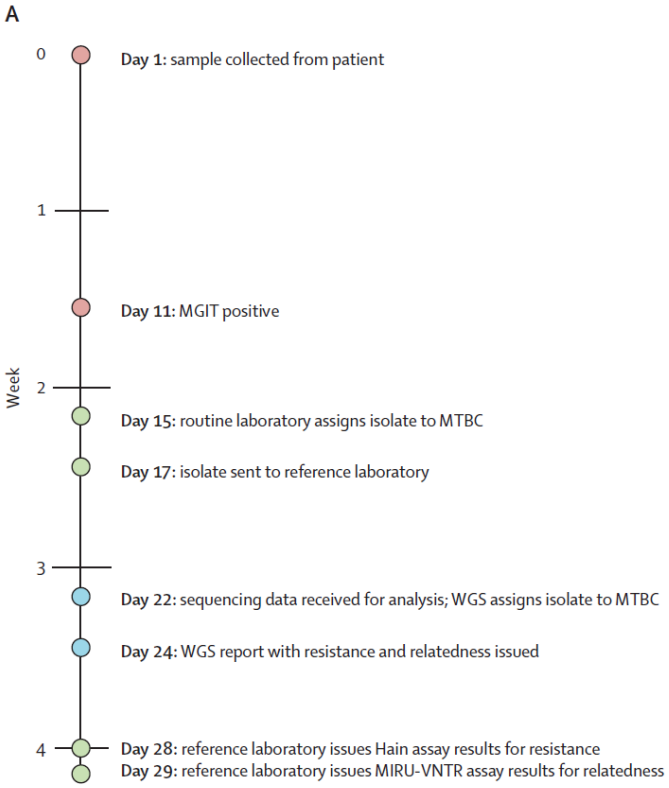
Progress

Lowenstein Jensen medium

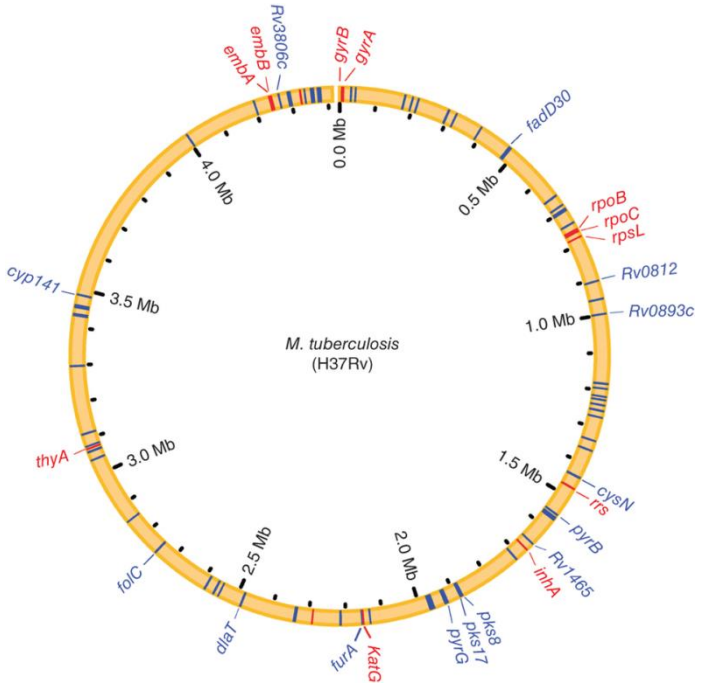
Sequencing

UK wide Whole Genome Sequencing

- Replacing MIRU VNTR in PHE Reference Laboratories Result within 5 working days of ***positive*** culture
- Drug resistance profiling including pyrazinamide and streptomycin
- Relatedness/ cluster data potential

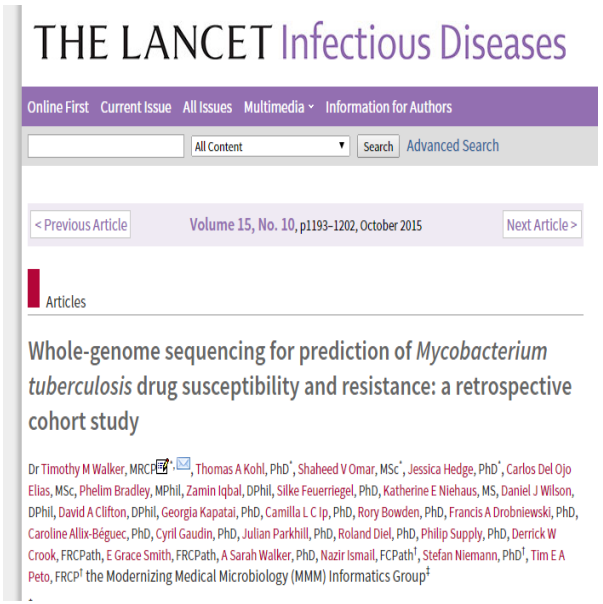


Pankhurst L et al. Lancet Respir Med 2016; 4: 49–58



Zhang H et al. Nature Genetics 45, 1255–1260 (2013)

Whole Genome Sequencing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Articles

Whole-genome sequencing for prediction of *Mycobacterium tuberculosis* drug susceptibility and resistance: a retrospective cohort study

Dr Timothy M Walker, MRCP, Thomas A Kohl, PhD, Shaheed V Omar, MSc, Jessica Hedge, PhD, Carlos Del Ojo Elias, MSc, Phelim Bradley, MPhil, Zamin Iqbal, DPhil, Silke Feuerriegel, PhD, Katherine E Niehaus, MS, Daniel J Wilson, DPhil, David A Clifton, DPhil, Georgia Kapatai, PhD, Camilla L C Ip, PhD, Rory Bowden, PhD, Francis A Drobniewski, PhD, Caroline Allix-Béguec, PhD, Cyril Gaudin, PhD, Julian Parkhill, PhD, Roland Diel, PhD, Philip Supply, PhD, Derrick W Crook, FRCPath, E Grace Smith, FRCPath, A Sarah Walker, PhD, Nazir Ismail, FCPath, Stefan Niemann, PhD, Tim E A Peto, FRCP the Modernizing Medical Microbiology (MMM) Informatics Group


Journal List > J Clin Microbiol > v.53(7); 2015 Jul > PMC4473240

Journal of Clinical Microbiology

JCM Article | Journal Info. | Authors | Reviewers | Permissions | Journals.ASM.org

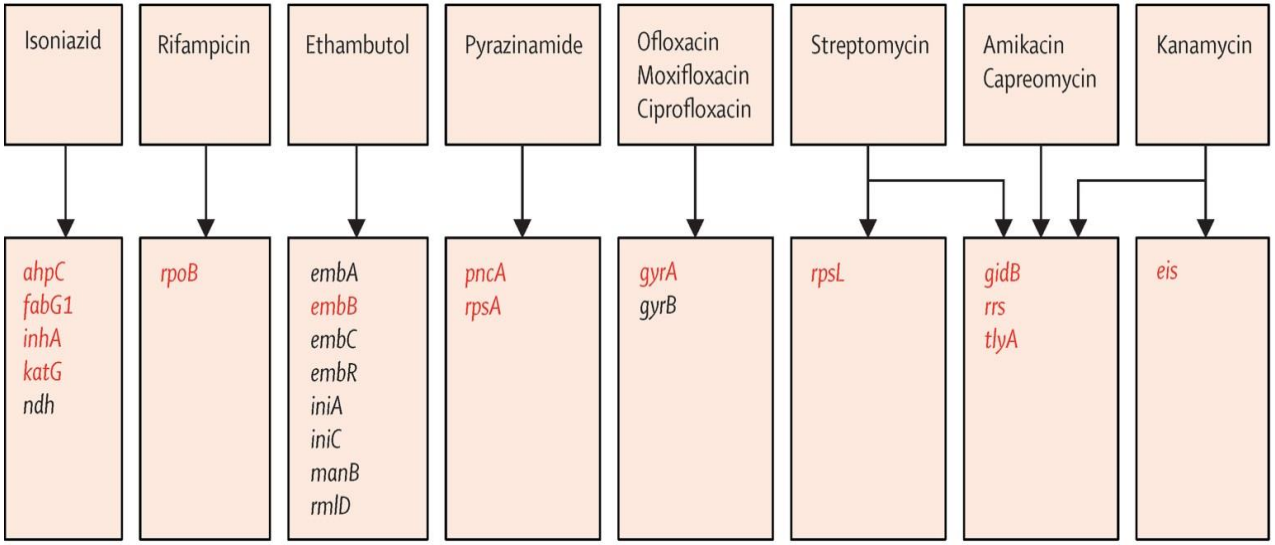
J Clin Microbiol. 2015 Jul; 53(7): 2230–2237. PMCID: PMC4473240

Published online 2015 May 13. doi: 10.1128/JCM.00486-15

Rapid Whole-Genome Sequencing of *Mycobacterium tuberculosis* Isolates Directly from Clinical Samples

Amanda C. Brown, Josephine M. Bryant, Katja Einer-Jensen, Jolyon Holdstock, Darren T. Houniet, Jacqueline Z. M. Chan, Daniel P. Depledge, Vladyslav Nikolayevskyy, Agnieszka Broda, Madeline J. Stone, Mette T. Christiansen, Rachel Williams, Michael B. McAndrew, Helena Tutill, Julianne Brown, Mark Melzer, Caryn Rosmann, Timothy D. McHugh, Robert J. Shorten, Francis Drobniewski, Graham Speight, and Judith Breuer

G. A. Land, Editor



- Within 5 days of culture positive sample
- Accurate sequencing of *M.tuberculosis* genome directly from uncultured sputa possible
- Identification of resistance mutations offers directed treatment of MDR-TB

2016 – 2017 Birmingham data
82 consecutive culture positive samples

- Time from sample to WGS result (days)
  - Fully sensitive Mean 28.7 (SD 17.4) Median 26 (IQR19.5-34)
  - Resistant Mean 37.8 (SD 21.9) Median 38 (IQR21.5-45)
- Time from sample to phenotypic result (days)
  - Fully sensitive Mean 54.6 (SD 24.6) Median 47 (IQR37-72)
  - Resistant Mean 86.5 (SD 38) Median 74.5 (IQR58-118)

Data courtesy Dr Martin Dedicoat

2014 versus 2018

Time to result by sample type (days) 2014 phenotypic vs 2018 WGS

- BAL 44.2 (SD 11.8) Vs 23.1 (SD 3.8)
- Sputum 66.1 (SD 41.9) Vs 30.7 (SD 21.8)
- Lymph Node 74.9 (SD 37.5) Vs 40.3 (SD 10.9)

Birmingham data courtesy Dr Martin Dedicoat

Relatedness

- 0-5 SNPs difference between strains, investigate epidemiology, most probably linked
- 5-12 SNPs may be linked
- >12 SNPs less likely to be linked

Whole-genome sequencing to delineate *Mycobacterium tuberculosis* outbreaks: a retrospective observational study

Timothy M Walker, Camilla L C Ip*, Ruth H Harrell*, Jason T Evans, Georgia Kapatai, Martin J Dedicoat, David W Eyre, Daniel J Wilson, Peter M Hawkey, Derrick W Crook, Julian Parkhill, David Harris, A Sarah Walker, Rory Bowden, Philip Monk†, E Grace Smith†, Tim E A Peto†*

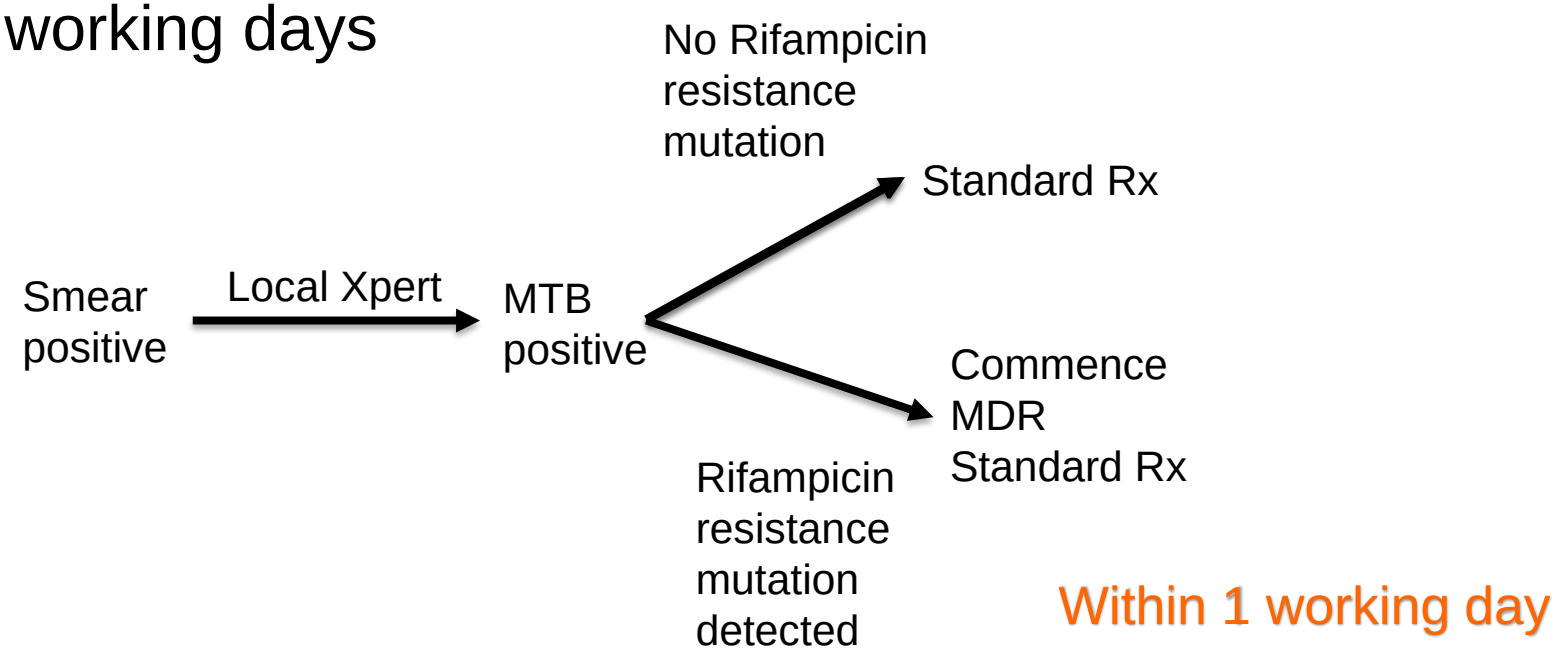
Assessment of *Mycobacterium tuberculosis* transmission in Oxfordshire, UK, 2007–12, with whole pathogen genome sequences: an observational study

Timothy M Walker, Maeve K Lalor, Agnieszka Broda, Luisa Saldana Ortega, Marcus Morgan, Lynne Parker, Sheila Churchill, Karen Bennett, Tanya Golubchik, Adam P Giess, Carlos Del Ojo Elias, Katie J Jeffery, Ian C J W Bowler, Ian F Laurenson, Anne Barrett, Francis Drobniewski, Noel D McCarthy, Laura F Anderson, Ibrahim Abubakar, H Lucy Thomas, Philip Monk, E Grace Smith, A Sarah Walker, Derrick W Crook, Tim E A Peto, Christopher P Conlon**

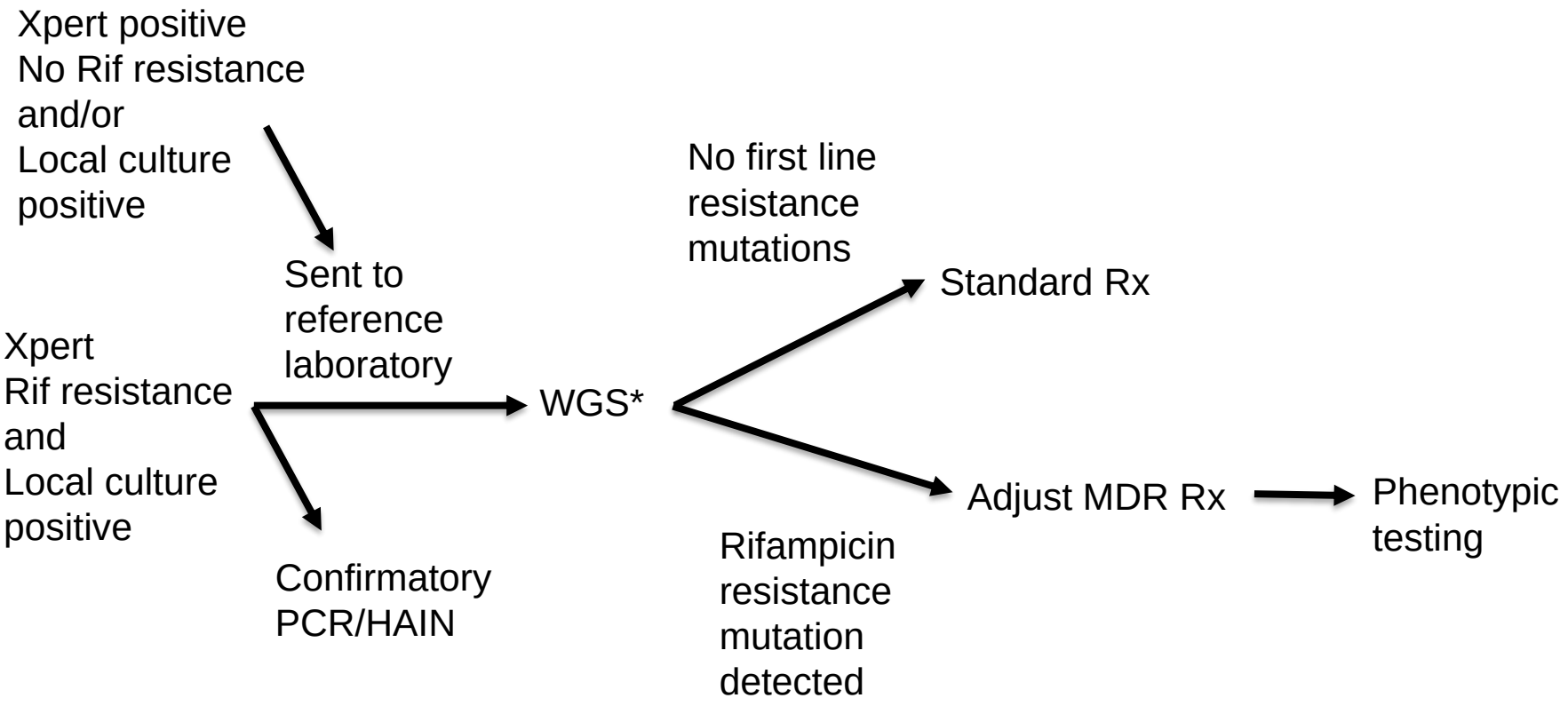
Still need local teams to examine social networks etc

Synergistic Approach Xpert® MTB/RIF to WGS

- Xpert MTB/RIF (Xpert) rapid and affects immediate management issues
- Xpert MTB/RIF locally available versus reference laboratory
- WGS still currently dependent on culture positivity then further 5 working days



Phase 2 - on culture (weeks)



*WGS turnaround time from receipt of positive cultured sample = 5 working days

Summary

- Rapid PCR
  - Greater sensitivity than smear – clinically useful
  - Case for use in all smear positive cases
  - Increasing role in EPTB (ITLN/CSF etc)
- WGS
  - Sensitivity results more rapid
  - Drug sensitivity prediction reliable, sensitive and specific
  - Iterative learning - unknown calls likely to decrease
  - Unexpected transmission may be detected
  - Relatedness results timely and useful for public health action
  - ?reduce unnecessary screening
  - ?redundancy of phenotypic testing

Acknowledgements

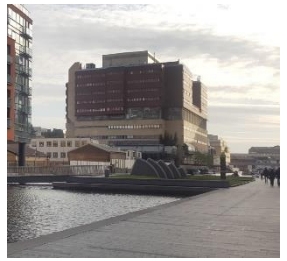
Charlotte Anderson

Martin Dedicoat

Oliver McManus

Al Story

Inis Hormann



NIHR HPRU in Respiratory Infections

NHS National Institute for Health Research

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